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FATAL HOOKWORM DISEASE IN INFANCY AND CHILDHOOD ON GUAM *

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From January 26th through August 9th, 1945, 50 post-mortem examinations were performed on children of 4 years of age and under in the U.S. Naval Military Government Hospital No. 203 on Guam, the Marianas Islands. In 21 instances, the primary cause of death was hookworm disease with its attendant complications. The next most frequent cause of death was tuberculosis, encountered in 5 cases. This rather startling and unique experience seems ample justification for adding another report to the already extensive literature on the subject of hookworm disease. Furthermore, these cases afford a clear view of some of the various mechanisms of the disease which nonfatal cases fail to show.

During the 6½ months which comprise the period of this study, life on Guam for the natives was, indeed, at a low level. Three years of Japanese occupation had so reduced the food supply that nutritional deficiencies, such as the avitaminoses and, more especially, hypoproteinemia, were quite frequent. The battle for possession of the island in the fall of 1944 reduced most of the permanent homes to mere rubble, and by the first of the new year the majority of the native families were living in small one-room huts which were little more than lean-tos. Sanitary facilities were most primitive and often nonexistent. The shallow pits employed as receptacles for excreta were filled to overflowing by the frequent heavy rains. Bathing and washing facilities were difficult to procure both because of excessive crowding in the hastily constructed villages and an inadequate water supply. Clothes, therefore, were soiled and worn for days without being changed. Under such conditions of existence, it is not surprising that nearly 100 per cent of the population was infected with hookworms and had other intestinal parasitism, such as ascariasis and trichuriasis. Of course, as

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sanitary conditions rapidly improved in the latter months of this study, both the incidence and severity of these parasitic infections dropped precipitantly.

Weather conditions on Guam are such as to make hookworm infection a constant threat. The year-round tropical heat and the abundant rainfall meet the conditions necessary for the maturation of hookworm ova into infective filariform larvae. Heavy vegetation affords the requisite shade for this developmental process. Yet 13 of the 21 children with fatal hookworm disease, who comprise the present series, were of an age (under 16 months) which almost precluded acquisition of the infection by the usual method of skin contact with contaminated soil. One of the more puzzling features of the disease was the extreme youth of the victims, but a plausible explanation for this has recently been found. Loughlin and Stoll [1] of this Research Unit have discovered that wearing apparel, and especially bed clothes, when contaminated with infective fecal matter and allowed to remain damp and unwashed for some days, will harbor appreciable numbers of filariform hookworm larvae. They have actually recovered viable larvae from these materials in homes within villages from which our fatal cases originated.

Two factors, in addition to the youth of the patients, were of prime etiologic importance in the production of the fatal hookworm disease. One, already briefly mentioned, was the general state of malnutrition coincident with the Japanese occupation and the subsequent destruction when Americans reoccupied the island. At 3 years of age, the children were still breast fed and received very little supplementary feeding. Green vegetables, meats, and fresh fruits were lacking in the diet. The high incidence of childhood tuberculosis perhaps attests the generalized malnutrition, and, in addition, 2 cases of fatal rickets were discovered in infants during this period. It is, therefore, reasonable to assume that the universal hypoproteinemia was as much the result of antecedent poor nutrition as of the hookworm infection itself.

The other factor was concerned with the species of parasite producing hookworm disease on Guam. Many writers [2,3] have emphasized the greater virulence of *Ancylostoma duodenale* as compared to *Necator americanus*. In view of this, Lieut. N.R. Stoll, H(S), U.S.N.R., undertook an investigation of the species of hookworms found in 11 of the 21 individuals necropsied at the Military Government Hospital. He also examined worms recovered from 7 other infants in the same hospital after anthelmintic treatment. From both sources he obtained a total of 982 hookworms and found that 93 per cent were *Ancylostoma duodenale*.[4] This was the sole species in 13 of the 18 cases. It should be mentioned that not a single *Ancylostoma braziliense* was discovered.

Clinical Histories

The data obtainable from the clinical records of the 21 fatal cases of hookworm disease are woefully inadequate in many respects. This was due to several reasons, not the least important of which were the unsettled conditions on the island at the time during which the study was made. More important, however, was the unexpected suddenness of the fatal termination of the illness in the patients who died in the hospital as well as in their homes. Frequently, there was little or no time in which to make the necessary physical and laboratory examinations. The available clinical data are summarized in Table I.

There was no predominance of the specific infection in female children as compared to male. Although it was realized in a number of instances that hookworm disease was the underlying condition, signs of a terminal infection, such as pneumonia, obscured this fact and led to a faulty evaluation of the primary and secondary causes of the illness. The short period of time during which most of the patients were under observation often precluded accurate differential diagnosis. It must be admitted, too, that the several physicians under whose observation these patients came were not fully aware of the possibility of hookworm disease assuming such a fulminating and fatal course.

It was difficult to determine with accuracy the duration of the terminal illness, and in 8 instances even an approximation could not be made. For the most part, these were the children who died at home. Careful inquiry of the respective families failed to elicit more than the observation that the child had been pale "for some time" but was not considered alarmingly ill. In most instances where the duration of the terminal illness was succinctly specified, it merely indicated when the condition took a decided turn for the worse.

The unusual findings on laboratory examination of cases 4 and 8 need further comment. Verification was made of both the red and white blood cell counts in the former case, and a repeated count of the leukocytes after 8 hours disclosed 98,000 cells with essentially similar differential values. The surprisingly high number of lymphocytes (92 per cent) in case 8 may be related to the nonsuppurative interstitial pneumonia found at necropsy.

Post-mortem Findings

Those circumstances which hindered the acquisition of adequate clinical data in the fatal cases of hookworm disease fortunately did not interfere with complete post-mortem examinations. A summary of the findings at necropsy is presented in Table II.

Summary of Clinical Data

Case no.	Sex	Age	Clinical diagnosis	Duration of terminal illness	Admission temperature	Pallor	Diarrhea	Vomiting	Cough
		(months)							
1	F	11	?	8 hours	100.5°–107°F.	6 mos.	–	–	–
2	M	3	Hookworm disease	5 days	99.5°	+	–	–	+
3	M	7	Pneumococcal meningitis	7 days	99°–103°	+	–	–	–
4	M	6	?	14 hours	104.5°	+	–	–	–
5	F	10	?	8 hours	101.5°	1 wk.	–	–	+
6	F	11	Hookworm disease	1 day	102.4°		4 days	+	–
7	F	12	Otitis media	2 wks.	98.6°	+	3 days	–	–
8	M	7	Hookworm disease; pneumonia	4 hours	100.0°	1 mo.	+	+	
9	M	8	Hookworm disease; pneumonia	1 day	100.0°–107°	2 wks.			+
10	F	24	Bronchopneumonia		101.0°	+			
11	M	7	? (Died at home)						
12	F	36	Bronchial asthma	18 hours	99.5°–103°		–	–	2 wks.
13	F	48	Peritonitis	12 hours	101.5°		2 days	+	–
14	M	14	Lobar pneumonia	13 hours	101.4°	+	–	–	2 days
15	F	24	? (Died at home)						
16	M	24	? (Died at home)						
17	M	36	? (Died at home)						
18	F	36	Peritonitis; pneumonia	4 hours	107.0°	+	–	+	+
19	F	16	? (Died at home)						
20	F	48	? (Died at home)						
21	M	8	Hookworm disease		104.0°	1 mo.		+	

Listed under malnutrition are 6 cases which represented rather severe degrees of physical underdevelopment and emaciation. For example, a child of 3 years of age, case 12, appeared hardly older than a 1-year-old child. With but 2 exceptions, the remaining 15 children were borderline cases of poor nutrition.

The diagnosis of anemia was based on several factors. One was the pale appearance of the mucous membranes and of the skin. The latter, because of the natural cutaneous pigmentation of the native Chamorro, had a striking "café au lait" color in the presence of anemia. More important, however, was the pale pink color and almost watery consistency of the blood. Also, samples of bone marrow removed from the sternum, ribs, vertebrae, and femur were pale and hypoplastic.

Accumulations of excessive amounts of fluid in the subcutaneous tissues, abdomen, chest, and pericardium were associated with one or more of the following findings: malnutrition, anemia, and cardiac dilatation. In 3 instances where there were such fluid accumulations in the presence of cardiac hypertrophy and dilatation, there was also evidence of congestive heart failure. This was indicated by the presence of chronic passive congestion in the lungs, liver, and spleen. One of the remarkable features presented by 7 of the fatal cases was the cardiac

TABLE

Clinical Data of Fatal Hookworm Disease

Physical examination			Laboratory examinations						
			Stool	Blood					
Lungs	Heart	Abdomen	Hookworm ova	Red blood cells	Hemoglobin	White blood cells	Segmented forms	Lymphocytes	Eosinophils
							(%)	(%)	(%)
Râles		Distention							
—		—	+	1,920,000	35%	10,850	70	19	9
Rapid respiration	Enlarged	—		2,270,000	7gm.	45,850	74	18	
Dyspnea				980,000	15%	102,900	40	32	12
			+						
			+	1,940,000	30%	14,000	64	18	16
Consolidation					1.5gm.	13,900	8	92	
Grunting respiration					2.5gm.	22,640	37	61	
Consolidation	Enlarged		+		5.0gm.	12,200	60	30	4
Dyspnea;cyanosis									
Râles		Soft							
Dullness	Tachycardia	Soft							
Irregular resp.	Tachycardia	Distention							
		Rigid	+	2,820,000	6.0gm.	10,900	55	34	4

enlargement. This was due to chronic dilatation alone in 4 instances and to both hypertrophy and dilatation in 3. The myocardium was invariably pale and had the yellow mottling associated with anemia. The chambers were greatly enlarged; the papillary muscles were prominent from hypertrophy but were also flattened (Fig. 1).

It has already been mentioned that a terminal febrile illness frequently confused the clinical picture and caused the underlying hookworm infection to be overlooked. In 7 instances, the terminal infection involved the lungs in a nonsuppurative interstitial inflammatory process indistinguishable from a viral pneumonia. In 3 additional cases there was an ordinary bronchopneumonia, with the inflammatory leukocytic exudate dispersed as small foci which were often in peribronchiolar locations. Another patient, case 14, had pneumococcal lobar pneumonia with fibrinopurulent pleurisy. Case 1 had a widespread hemorrhagic pneumonia of undetermined etiology. Finally, there were 2 instances of bronchial involvement. One was an ordinary purulent tracheobronchitis (case 13) and the other (case 12) was a bronchiolitis with plugs of mucus, hyalinization of the basement membrane, and peribronchiolar eosinophilic infiltration. It was the latter case which was diagnosed clinically as bronchial asthma, and the anatomic findings

seemed to support this diagnosis. Thus, 14 patients of this series had infections of the respiratory tract complicating hookworm disease.

Apart from inflammatory changes, the lungs were altered in other ways in 3 patients. One, case 15, had multiple fresh hemorrhages, and 2 others, cases 3 and 6, had fibrosis. The presence of various types of débris in the alveoli and in the capillaries of the alveolar walls in these and other cases suggested fragmented or disintegrating hookworm larvae. Accordingly, a careful search was made through many microscopic preparations of the lungs of these patients, but no well formed, identifiable larvae were found. Some of the débris, nevertheless, had the colorless, translucent appearance of larval sheaths. To permit comparison between the material found in the lungs of some of the patients and actual hookworm larvae, a cat was infected with *A. duodenale* by having many filariform larvae placed on the pads of its feet. At the end of 6 days, numerous parasites, as well as an inflammatory cellular reaction, were found in the pulmonary alveoli (Fig. 18). The débris which resulted from disintegrating larvae was closely similar to that seen in the human lungs, yet positive identification could not be made in the latter. The pulmonary fibrosis and the focal hemorrhages may possibly represent injuries which resulted when the larvae penetrated the alveolar septa to enter the acini.

Other serious infections obscured the underlying hookworm disease. In case 3, a 7-months-old male infant who had evidence of severe hookworm disease, there developed an acute otitis media which progressed to mastoiditis and terminated in pneumococcal meningitis. A female infant, 11 months old, case 6, developed a serious thrush infection of the pharynx. The outstanding inflammatory lesions, however, affecting 18 of the 21 cases, were the result of the hookworm infection itself and involved the jejunum and upper part of the ileum. In 6 of these cases, an extensive fibrinopurulent peritonitis had also occurred.

The involvement of the jejunum invariably began at the fossa of Treitz and extended down for a variable distance in the ileum, rarely affecting more than the first 50 cm. The duodenum was involved only once or twice to the extent of harboring a few hookworms. Macroscopic inspection of these parts of the small intestine disclosed all gradations of involvement ranging from little more than occasional petechiae to gangrene and extensive hemorrhage of the jejunum, with fibrinopurulent peritonitis, in the most severely involved cases (Fig. 2). The intestinal wall was greatly thickened, but in no instance was it actually perforated. Examination of the mucosal surface of the jejunum frequently disclosed many adult hookworms attached to the villi (Fig. 3). Petechial hemorrhages in the mucosa were found at sites

TABLE II

Summary of Necropsy Findings in Fatal Cases of Hookworm Disease

Case no.	Malnutrition	Anemia	Lymphadenitis	Eosinophilia in bone marrow	Anasarca	Hydrothorax	Hydropericardium	Ascites	Chronic passive congestion of viscera	Heart	Lungs	Intestines	Miscellaneous findings	Hookworms	Ascaris	*Trichuris trichiura*
1	+	+			+	+	+		+	Hypertrophy and dilatation	Hemorrhagic pneumonia	Acute enteritis		20+	–	–
2		+									Aspiration of gastric contents	Acute jejuno-ileitis; blood		99	–	–
3		+			+	+	+		+	Dilatation	Fibrosis	Focal jejunitis; blood	Otitis media; meningitis	197	–	–
4		+	+								Interstitial pneumonia	Petechiae and ulcers of jejunum	Focal hepatitis and fibrosis	60+	–	–
5		+	+				+				Aspiration of milk	Ulcerative jejuno-ileitis		200+	–	–
6	+		+								Interstitial fibrosis	Ulcerative jejuno-ileitis	Thrush pharyngitis	24	1	–
7		+	+					+	+	Hypertrophy and dilatation	Interstitial pneumonia	Ulcerative jejuno-ileitis		150+	1	–
8		+	+		+	+					Interstitial pneumonia	Acute jejuno-ileitis		200+	–	–
9 10		++	++		+					Dilatation	Edema	Acute jejunitis		250	–	–
11 12		++	+			+					Focal pneumonia	Petechiae in ileum	Acute peri-cholangiitis	50	–	+
											Acute bronchiolitis	Acute jejuno-ileitis		7	1	–
13 14	+	++								Dilatation	Tracheobronchitis	Gangrene of jejunum	Pulmonary emphysema and atelectasis	100+	3	++
											Diffuse pneumonia; pleurisy		Purulent peritonitis	75	31	++
15		+	+	+							Multiple hemorrhages	Ulcerative jejunitis		50+	–	–
16 17			++	+							Focal interstitial pneumonia	Necrotizing jejunitis	Purulent peritonitis	70+	27	–
												Suppurative jejunitis	Purulent peritonitis	20+	–	–
18 19		+		++	+	+		+		Hypertrophy and dilatation	Hemorrhagic pneumonia	Necrotizing jejunitis	Purulent peritonitis	100+	–	–
		+								Dilatation		Necrotizing jejunitis	Organizing peritonitis	43	1	48
20 21	+										Focal pneumonia Intermittent	Necrotizing jejunitis	Purulent peritonitis	200+ 50+	2 –	+ –

where parasites were no longer attached. Many worms were also found free in the fecal contents. In several instances, large quantities of partially changed blood were mixed with the feces. As a rule, the mesenteric lymph nodes of the most heavily infected patients were greatly enlarged, pale, and succulent.

On microscopic examination, the jejunum of every patient was infiltrated with polymorphonuclear neutrophilic leukocytes and with eosinophils in various proportions. Sometimes this cellular reaction was limited to the mucosa, at other times it extended to involve the submucosa, and at still other times it infiltrated the muscularis as far as the serosa (Fig. 4). Serial microscopic preparations of the jejunum disclosed pieces of mucosa clamped in the mouth parts of hookworms (Fig. 5). In some instances, the head of a parasite had evidently burrowed through the mucosa and penetrated the submucosa (Fig. 6). Superficial mucosal ulcerations, oftentimes exceedingly numerous, marked former sites of attachment of hookworms (Fig. 7). Almost invariably there was still evidence of bleeding in these ulcers, and the subjacent wall was heavily infiltrated with leukocytes. In a few instances, all layers of the intestinal wall contained enormous numbers of eosinophils (Fig. 8). The great thickness and leathery consistency of the jejunum were due in part to the cellular infiltration and in part to a peculiar edema which was most prominent in the submucosa (Fig. 9). Lymphocytes and neutrophilic leukocytes surrounded the submucosal lymphatics as evidence of an extensive lymphangiitis. All too frequently, sites adjacent to areas where the worms were attached were deeply ulcerated and secondarily infected (Fig. 10). This mechanism undoubtedly accounted for the frequent presence of abscesses in the submucosa (Fig. 11). The 6 cases of peritonitis had the most extensive lesions in the small intestine. Not only was the mucosa extensively ulcerated, but there were necrosis, hemorrhage, and leukocytic reaction in all coats (Figs. 12 and 14). Extensive thrombo-angiitis of the vessels in the submucosa accounted for part of the microscopic picture of gangrene. Large vessels, arterioles as well as venules, had necrotic walls even when they were not thrombosed (Fig. 13). Less involved portions of the gut often had zones of hyaline necrosis (Zenker's) of the muscle layers (Fig. 15).

Microscopic study of the attached hookworms themselves disclosed an interesting fact. Their intestinal canals contained bits of ingested mucosal tissue as well as leukocytes and erythrocytes. None of these cellular elements appeared to be altered by their passage from the buccal end of the parasite's intestinal canal to its anal orifice. In Figure 17

are portrayed such unaltered leukocytes and red blood cells in the terminal end of the intestine of a hookworm.

The greatly enlarged mesenteric lymph nodes had widely dilated marginal lymphatics and contained many macrophages filled with phagocytosed white and red blood cells and other débris. A conspicuous feature of all nodes was the presence of numerous eosinophils. Cells of the latter variety were also present in large numbers in the splenic pulp.

In 8 cases there were changes in the bone marrow which could have accounted, at least in part, for the severity of the anemia. These changes consisted of an eosinophilia that replaced the cells of the erythropoietic series (Fig. 16). Eosinophilic myeloblasts and myelocytes were present in large numbers, in addition to mature eosinophilic leukocytes. Conversely, there were few megakaryocytes and erythroblasts. As evidence of this deficiency in hematopoiesis on the part of the bone marrow was the presence of megakaryocytes in both the pulmonary capillaries and the spleen. In 2 of the cases, indeed, there was also evidence of erythrocyte formation in the liver.

Discussion

The combination of extreme youth of the patients, their malnutrition and neglect, opportunities for reinfection, and the virulent species of hookworm involved, namely *Ancylostoma duodenale,* accounted for the malignant form which the intestinal parasitism assumed in this series of cases. It seems fair to assume that the simultaneous infection with *Ascaris lumbricoides* and with whipworms (*Trichuris trichiura*) contributed but little to the fulminating course, especially since only 2 children had moderately heavy infections with Ascaris and only 5 of the children were infected with whipworms. The unusual penetrating proclivities of the hookworms in this series of cases, as shown in Figure 6, are suggestive of the behavior of *Ancylostoma braziliense.*[5] That this species of helminth had no part in the production of the intestinal disease seems apparent from the study undertaken by Lieut. Stoll to which reference has already been made.[4]

Most writers agree with Rhoads, Castle, Payne, and Lawson [6] that the anemia of hookworm disease is the result of blood loss. This occurs from the numerous bleeding ulcerations in the intestinal mucosa, as well as through the intestinal tracts of the attached hookworms. The presence of large quantities of blood in the intestinal lumina of our patients amply confirms this as a prime mechanism of the anemia. But the additional finding of eosinophilia in the bone marrow, with the

consequent interference of normal erythropoiesis, is evidence of the fact that the anemia of the more severe cases is not due alone to blood loss.

Summary

Twenty-one fatal cases of hookworm disease in early childhood on Guam were investigated.

Malnutrition, neglect, and poor sanitation were considered important factors in this condition.

It was suggested that bed clothes contaminated with filariform larvae of *Ancylostoma duodenale* were the means by which at least some of the children became infected.

Serious terminal infections, chiefly of the respiratory tract, often obscured the underlying hookworm disease.

The outstanding lesion in the condition was acute jejunitis, which progressed in some cases to produce widespread ulceration, hemorrhage, necrosis, and even gangrene of this part of the intestinal tract.

In 6 cases there occurred an extensive purulent peritonitis.

A prominent feature of the intestinal lesion was the infiltration of eosinophilic leukocytes.

Associated with severe anemia, 7 cases had cardiac hypertrophy and/or dilatation.

There was evidence indicating that the anemia was due in part to eosinophilic accumulations in the bone marrow which interfered with normal erythropoiesis.

Acknowledgment is made of the valuable assistance of Robert H. Jackson, Pharmacist's Mate, 2nd Class, U.S.N.R., in histologic technic.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

All photomicrographs were made from preparations stained with hematoxylin and eosin, unless otherwise indicated.

PLATE 207

FIG. 1. Case 19. Female infant, 16 months of age. Cardiac hypertrophy and dilatation. Weight of heart, 95 gm.

FIG. 2. Case 13. Gangrene of the jejunum with purulent peritonitis.

FIG. 3. Case 9. Numerous hookworms attached to the jejunal mucosa.

FIG. 4. Case 2. Infiltration of neutrophilic and eosinophilic leukocytes in all coats of the intestinal wall. × 70.

FIG. 5. Case 5. Hookworm with a piece of intestinal mucosa in its mouth. × 70.

FIG. 6. Case 12. Head of a hookworm impinging on edematous submucosa of the jejunum. × 70.

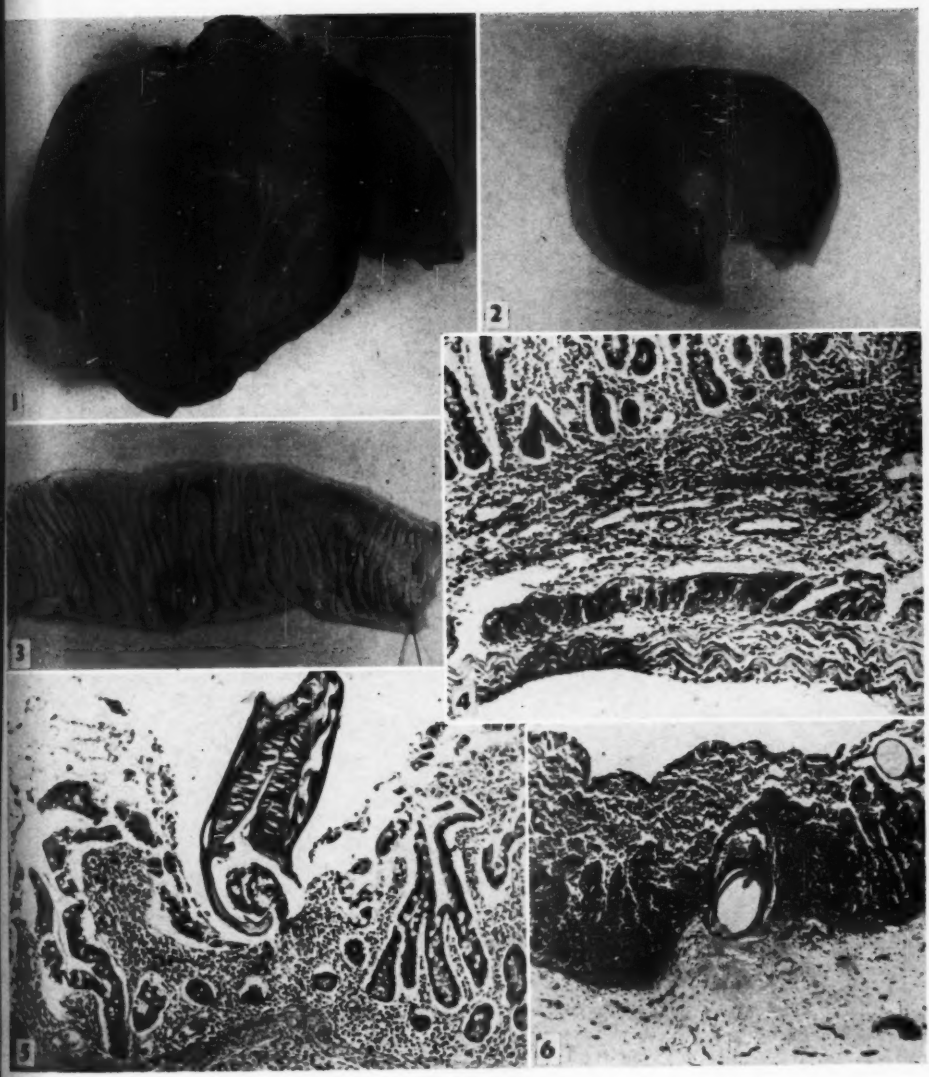

PLATE 208

FIG. 7. Case 19. Cross-section of a hookworm adjacent to superficial mucosal ulceration. Of note is the extensive leukocytic infiltration in the submucosa. × 80.

FIG. 8. Case 4. Cells in both the mucosa and submucosa of the jejunum are almost entirely eosinophils. × 80.

FIG. 9. Case 19. Massive edema and lymphangiitis in the submucosa of the jejunum. × 80.

FIG. 10. Case 14. Ulcerative lesion adjacent to the site of a hookworm attachment. × 80.

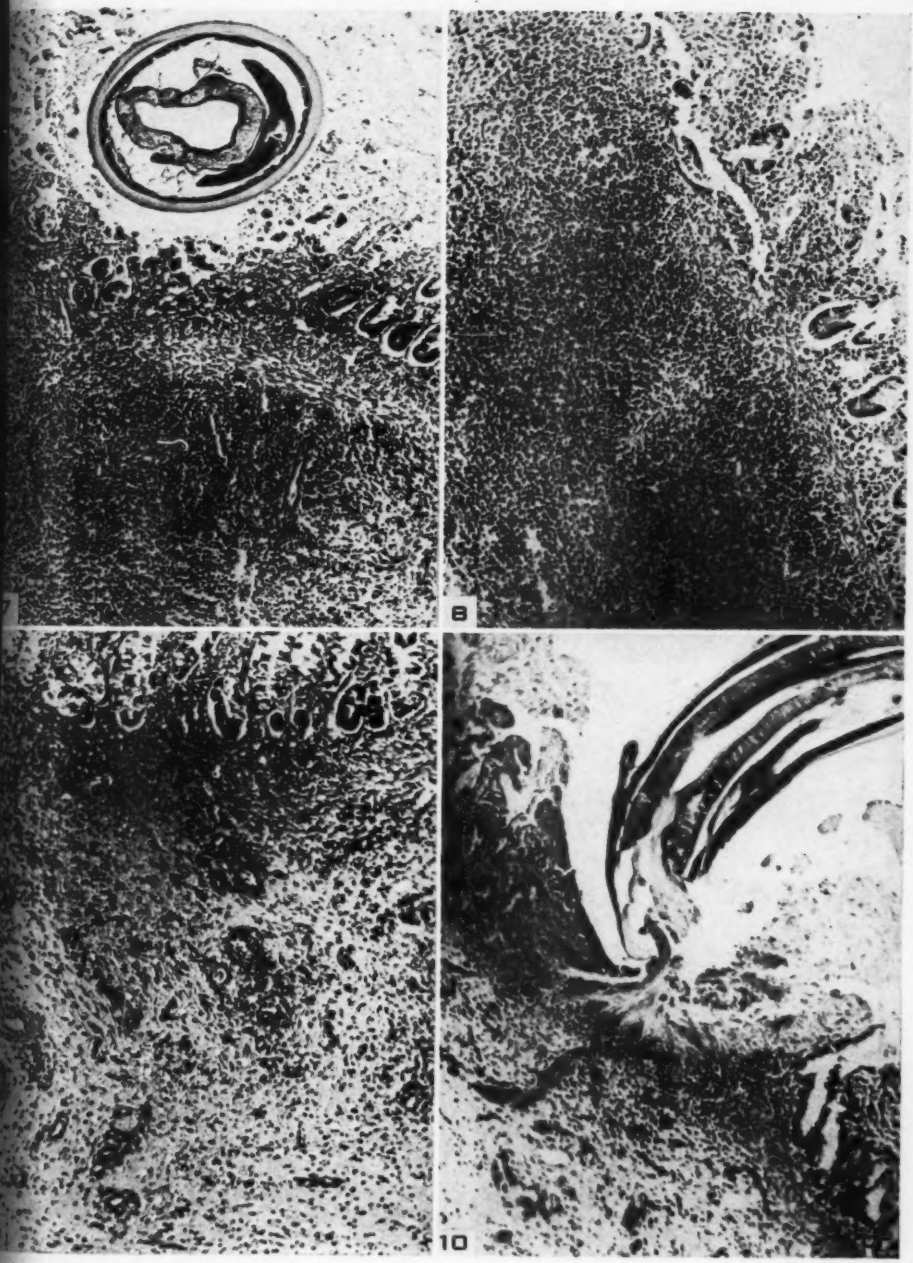
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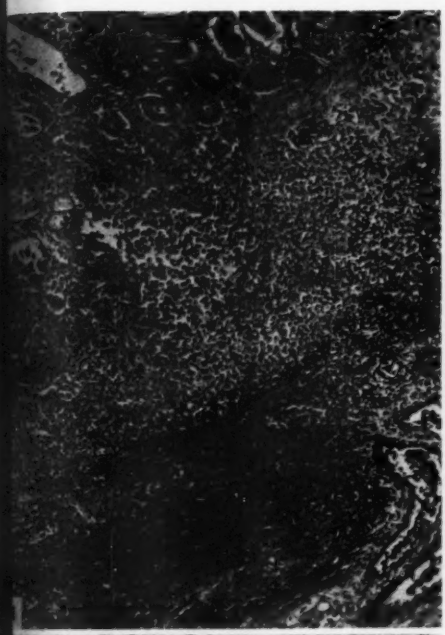
PLATE 209

FIG. 11. Case 6. Abscess in the submucosa of the jejunum. The dark area on the left represents hemorrhage. × 80.

FIG. 12. Case 15. Mucosal ulceration, hemorrhage, leukocytic infiltration, and thrombo-angiitis in the jejunum. × 80.

FIG. 13. Case 20. Necrosis of the walls of an arteriole and a venule in the submucosa of the jejunum. × 285.

FIG. 14. Case 13. Necrotizing and suppurative jejunitis. × 80.



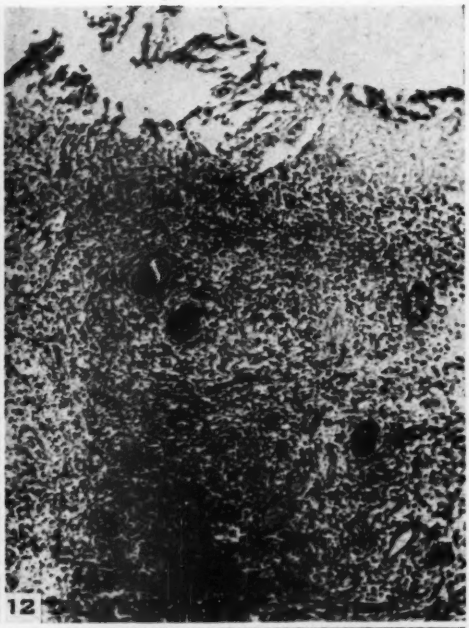

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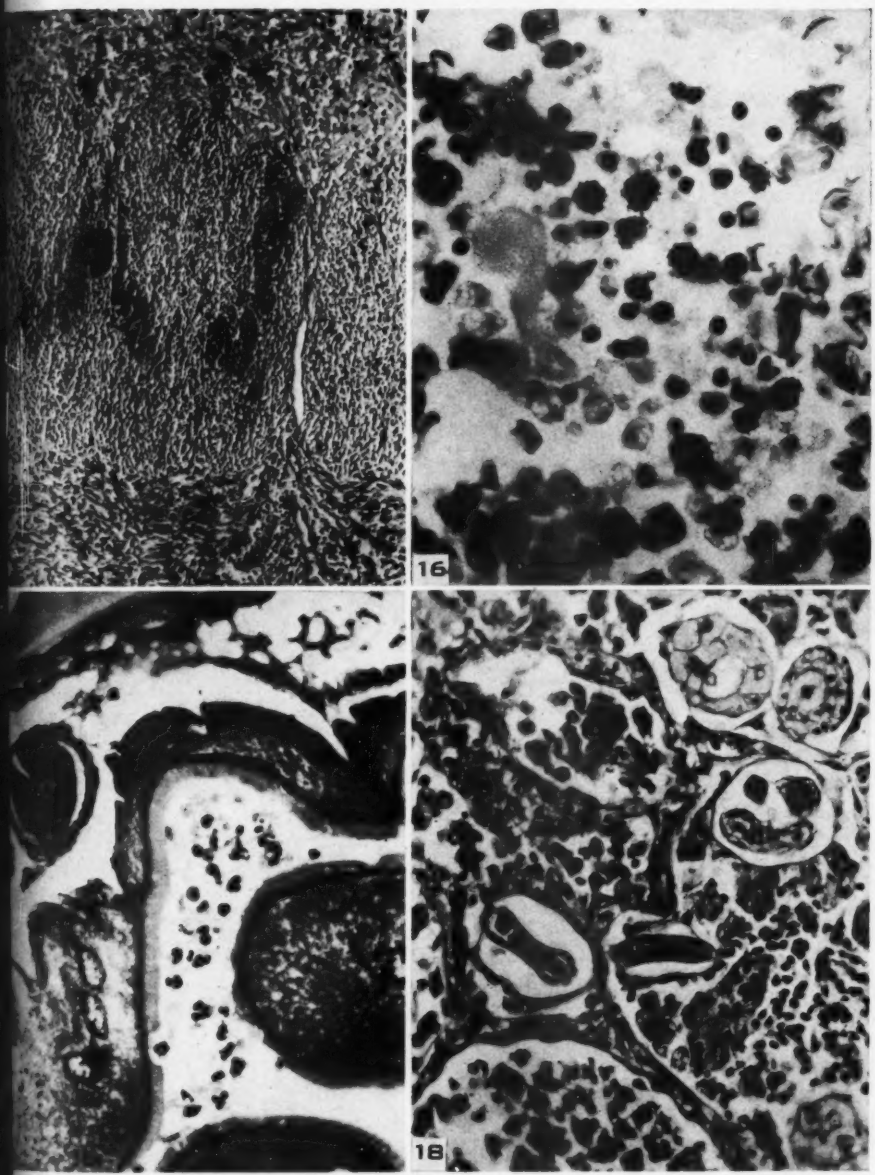
PLATE 210

FIG. 15. Case 15. Zenker's necrosis in the muscle layer of the jejunum. × 80.

FIG. 16. Case 13. Eosinophils (with large granules) in the bone marrow. Giemsa's stain. × 735.

FIG. 17. Case 19. Intact erythrocytes and leukocytes in the lower intestinal tract of a hookworm. × 285.

FIG. 18. Lung of a cat infected with filariform larvae of *Ancylostoma duodenale* 6 days previously. Of note are the larvae, inflammatory cellular exudate, and débris in the pulmonary alveoli. × 285.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EXPERIMENTS ON THE SPREAD OF NEOPLASTIC CELLS THROUGH THE RESPIRATORY PASSAGES*

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Little is known about the spread of tumors by way of the respiratory passages. The presence of mucus and the ciliary motion of the lining epithelial cells are believed to oppose the spread of neoplastic cells. Willis [1] stated that no experimental work had been reported on the inoculability of the lungs to tumors by way of air passages. He cited numerous alleged instances of implantation metastasis by way of air passages, but concluded that most of these are susceptible to other explanation. According to Willis,[1] Moxon (1869) was the first to report on inhalation metastases from an esophageal cancer, and Godlee from carcinoma of the tongue. However, the occurrence of intrapulmonary implants in a child from laryngeal carcinoma and from an adenoacanthoma in a young man can scarcely be doubted. There are numerous reports in the literature on contralateral metastases of pulmonary growth, all of which have been critically reviewed by Willis.

It was, in part, an accidental observation; in part, curiosity, that led us to undertake to verify the possibility of the spread of tumors by way of the respiratory passages. A study of respiratory viruses in mice made with Dr. P. deGara and Miss Dorothy Kley necessitated intranasal instillation of material containing cells from lung tumors. Unexpectedly this resulted in the development of lung tumors. Recently we made post-mortem studies of a patient who had a slowly progressing malignant adenoma of the trachea, and noted tumor nodules surrounding and stenosing several bronchioles in addition to the usual perivascular lymphatic metastases. A specimen taken for biopsy 4 years before death showed squamous metaplasia of the normal ciliary epithelium, which may have been sufficient to break down the barrier to penetration of foreign particles to the alveoli. Tumor cells which reach the alveoli readily proliferate there, as attested by the occasional findings of massive intra-alveolar growth in metastatic tumors of the lung.

In the experiments to be described a few droplets of a suspension of neoplastic cells were instilled into the nostrils of young healthy mice under light ether anesthesia. In several instances the instillation was repeated after 5 or 24 hours, as indicated in Table I. All mice used

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were highly susceptible to subcutaneous implantation of the tumor cells.

The results summarized in Table I indicate that the respiratory surfaces of a healthy animal did not bar dissemination of tumor cells, although in many mice all tumor cells introduced were destroyed. Intercurrent infection was slight, if any.

The course and behavior of the neoplastic cells after intranasal introduction varied greatly:

(a) The carcinoma cells (strain 387) produced progressively growing tumors in both lungs, bringing about death by displacement of lung

TABLE I

Results of Intranasal Instillations of Neoplastic Cells

Experiment no.	Neoplasm		No. of injections	No. of mice		Length of life after inoculation	
	Type	Number		Injected	Positive	Positive	Negative
						(*days*)	(*days*)
1	Carcinoma of lung	387	1	6	2	K*15, D29	K15–60
			1	6	2	K15, D21	K15–56
2	Carcinoma of lung	387	1	5	1	D*19	D7, K21–35
			1	3	2	D14–24	K31
3	Carcinoma of lung	387	2	5	3	D19–36	K51, K51
			2	6	2	D18–21	D14–32
4	Carcinoma of lung	387	2	5	5	D14–20	K20
5	Carcinoma of lung	387	1	7	1	D20	K104
			2	7	6	D19–57, K23	K23
6	Lymphoid leukemia	559	2	9	9	K17, D18–22	
7	Lymphoid leukemia	1329	1	3	1	D33	K86
8	Lymphoid leukemia	975	2	5	5	K24–29	
9	Myeloid leukemia	1394	2	5	4	D33–44, K42	K42
10	Myeloid leukemia	1446	1	5	1	K76	K76
11	Myeloid leukemia	1446	2	6	6	D34–37, K38	

* K = killed, D = died.

parenchyma (Figs. 1 and 3). At the time of death, gross examination disclosed metastases in regional lymph nodes only.

(b) The other extreme of invasiveness is represented by malignant lymphocytes of strain 559 which produced systemic disease without leaving any mark at the point of entry into the circulation.

(c) With respect to invasiveness all other malignant cells studied were between the above extremes. They produced massive peribronchial infiltrations with small or minute tumor-like nodules in the lungs (Figs. 4 to 7), particularly in hilar and apical regions, and moderate or extensive involvement of the draining nodes. In many instances the pulmonary infiltrations extended beyond the pleura, as shown in Figure 7, causing massive pleural effusion and occasionally massive pericardial infiltrations as shown in Figure 8. Death was usually caused by generalized leukemia with characteristics peculiar to the strain (*e.g.*, Fig. 9).

Many neoplastic cells doubtless perish after intranasal introduction

and occasionally all cells perish (Table I). Ability to survive under the circumstances as well as the degree of invasiveness are inherent characteristics of the cells.[2]

All but one of the strains used in these experiments produced a typical monomorphous invasive growth in the lungs and elsewhere of cells of the type named. Strain 1446 was an exception. This strain produced, in places, a fairly uniform tumor-like proliferation of myeloid cells (Fig. 10). Frequently, however, retrogressive changes occurred with fibrosis and accumulations of lymphoid cells, and in draining hilar lymph nodes a granulomatous reaction with multinuclear giant cells developed. This phenomenon needs further study. When death occurs with generalized leukemia of this strain there is usually massive infiltration in the liver (Fig. 9) and exsanguinating hemorrhage, probably caused by prothrombin deficiency.

Comment

That neoplastic cells appear in the sputum is now well established [3] although little use is being made of the diagnostic potentialities of such observations. Very recently, Papanicolaou has been highly successful in demonstrating malignant cells in the sputum of patients.[4] The experiments here described indicate that neoplastic cells in the sputum or in alveoli may lead to bronchiogenic spread.

The sequence of events is determined by the number and character of the malignant cells as well as by the state of the respiratory surfaces. Lymphoid cells of strain 559 penetrated the respiratory surfaces, producing systemic leukemia with no gross pulmonary infiltrations, and killed mice but a few days more slowly than when introduced directly into the circulation. It is now well known that the manifestations of leukemia depend in part on the invasive character of leukemic cells, in part on their portal of entry (site of origin).[2] This is well illustrated by the experiments here described.

Chloroleukemia, strain 1394, has not produced grossly detectable pulmonary infiltrations following intravenous or subcutaneous introduction into hundreds of mice, but it produced massive tumor-like infiltrations in the lungs following intranasal injection. From the lungs the malignant promyelocytes spread to the hilum and distant organs, killing the mice with either generalized myeloid leukemia or thoracic myelomatosis predominant.

None of the leukemic strains used in this study produced peribronchial infiltrations in the lungs following intravenous introduction. The characteristic feature of the pulmonary changes in the animals that received intranasal instillations of neoplastic cells, on the contrary,

consisted of massive, often tumor-like, peribronchial infiltrations. The figures shown amply illustrate the outstanding feature of these changes; namely, the preservation of the bronchial epithelium in most places and its lifting up from the underlying structures by the neoplasm. No conspicuous ulceration of the mucosa of the respiratory tract was seen in the sections examined, and the site of penetration of the malignant cells was not evident.

Summary

Epithelial surfaces of the respiratory tract are not safe barriers against the dissemination of neoplastic cells. Carcinoma cells instilled into the nostrils of lightly anesthetized mice reached the lungs and produced large tumors. Leukemic cells of a lymphoid strain similarly introduced produced fatal leukemia with no gross pulmonary changes. Leukemic cells of four other strains (two myeloid, and two lymphoid) produced tumor-like pulmonary infiltrations, with spread to regional lymph nodes terminating in generalized leukemia.

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4. Papanicolaou, G. Personal communication.

DESCRIPTION OF PLATES

All sections are from mice that received intranasal instillations of neoplastic cells. The sections were stained with hematoxylin and eosin. The magnifications given are approximate.

Plate 211

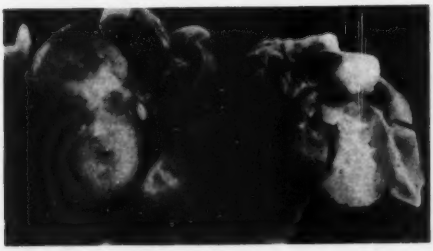
Fig. 1. Lungs of two mice that died following intranasal instillation of cells of a tumor of the lung, strain 387. Slight magnification.

Fig. 2. Lungs of a mouse that died following intranasal instillation with leukemic lymphocytes of strain Akl 975, showing massive mediastinal lymphomatous infiltrations. Slight magnification. Dorsal view of both lungs.

Fig. 3. Infiltrating neoplasm in the lung of a mouse which died 19 days following intranasal instillation of lung carcinoma cells from strain 387. × 100.

Figs. 4 and 6. These photomicrographs are from a mouse that died 42 days following intranasal instillation of malignant myelocytes of strain 1394. × 100. In this and other pictures the bronchial mucosa is lifted away from the underlying submucosa by massive neoplastic infiltrations.

Fig. 5. Lung of a mouse which died 35 days following intranasal instillation of cells from myeloid leukemia, strain 1446. × 100.



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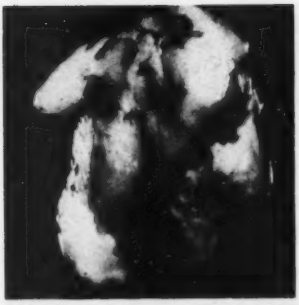

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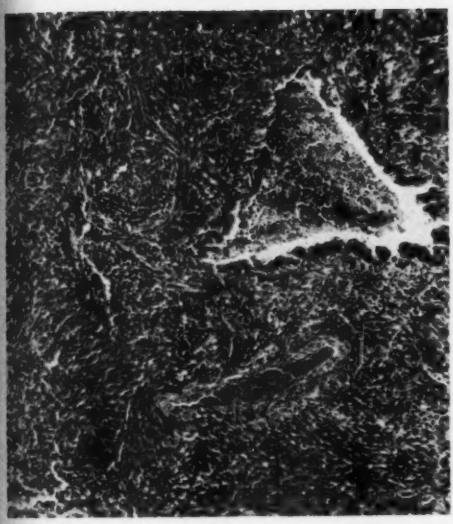

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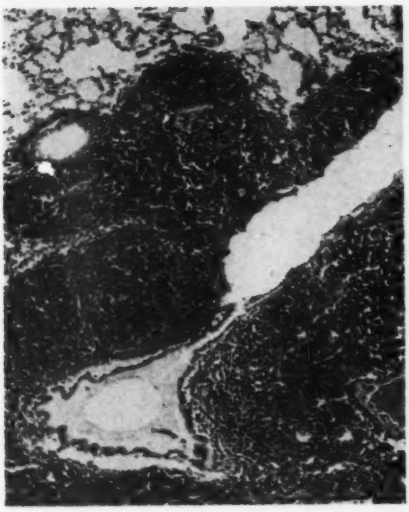


6

PLATE 212

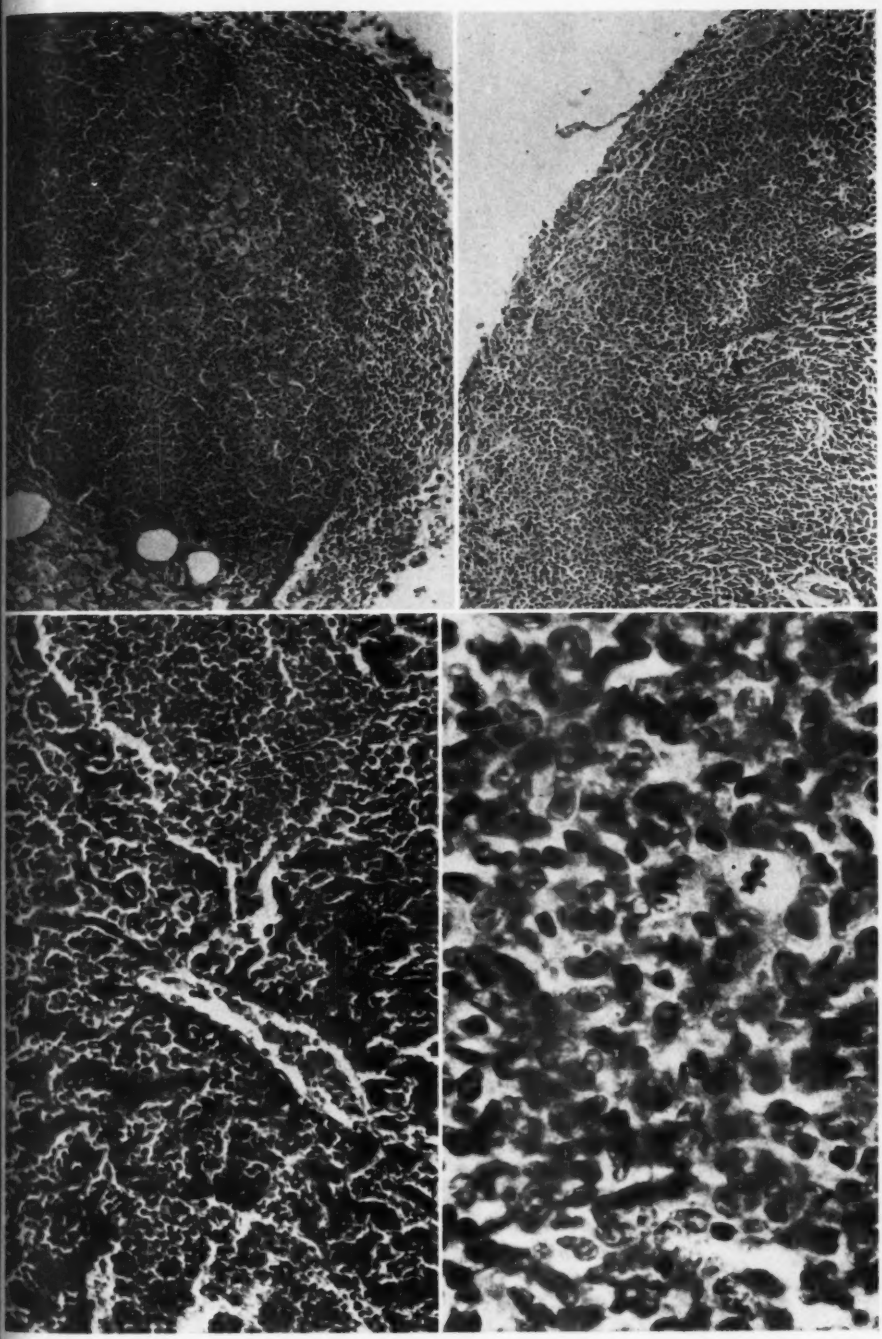
Figures 7 to 10 are of organs of mice which died following intranasal instillation with myeloid leukemia, strain 1446.

FIG. 7. A nodule of myelomatous infiltration in the lung of a mouse that died 37 days following intranasal instillation. There is central necrosis and extension of leukemic infiltration into the pleural space. × 100.

FIG. 8. Massive pericardial infiltration in the same mouse as shown in Figure 7. × 100.

FIG. 9. Massive leukemic infiltration in the liver of a mouse that died 34 days following intranasal instillation. × 180.

FIG. 10. Higher power view (× 900) of myelomatous mediastinal infiltration in the lung of a mouse that was killed 38 days following intranasal instillation. Of note are the mitotic figure and the pale-staining cells with doughnut-shaped or indented nuclei—characteristic myelocytes of the mouse.



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10

Furth Neoplastic Cells in the Respiratory Passages

HEMORRHAGIC DIATHESIS EXPERIMENTALLY INDUCED BY DEFICIENCY IN VITAMIN K

A Histopathologic Study *

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Since Dam's [1,2] studies on the sterol metabolism of chicks, which later on led to discovery of vitamin K (anti-clotting or anti-hemorrhagic vitamin), numerous articles dealing with the physiochemic, biochemic, physiologic, and therapeutic properties of vitamin K have been published.[3-17] However, the general pathology and especially the histopathology of vitamin K deficiency has been neglected.[18-21]

In a previous publication we reported the histopathologic findings in the central nervous system in the course of experimental vitamin K deficiencies in chicks and rats.[22] The purpose of the present paper is to present a detailed description of the general clinical observations and of the histologic changes in various tissues and viscera, and to discuss their relationship to other dietary deficiencies and to some similar findings in human pathology.

Experimental Material

In this investigation, 120 white Leghorn chicks and 90 albino rats were used.

Both chicks and rats (first and second generations) were divided into two groups. In one group the acute type, in the other, the subchronic and the chronic type of deficiency was investigated. All animals were fed with the following basic, modified, Dam-Schönheyder's vitamin K-free diet: casein,† 20 per cent; yeast,‡ 15 per cent; sugar, 62.3 per cent; salt mixture,§ 2.7 per cent; vitamins A and D in the form of cod-liver oil (Schieffelin), 4 cc. to 100 gm. of diet; vitamin E in the

* Received for publication, November 21, 1945.

† S.M.A. Corp. (Wyeth), vitamin-free casein as suggested by S. Ansbacher.

‡ Fleischmann's brewers' yeast containing the following vitamins: thiamine hydrochloride, 1–1.6 mg.; riboflavin, 4–6 mg.; niacin, 36–54 mg.; pyridoxine, 5–7.5 mg.; pantothenic acid, 12–18 mg.; and undetermined amounts of all other vitamins of B complex natural to yeast.

§ Salt mixture no. 351 prepared as indicated by Hubbell, Mendel, and Wakeman.[24] In a second series of experiments the formula was as follows: calcium carbonate, 543 gm.; magnesium carbonate, 25 gm.; magnesium sulfate, 16 gm.; sodium chloride, 69 gm.; potassium chloride, 112 gm.; potassium phosphate (di-basic), 212 gm.; iron phosphate, 20.5 gm.; potassium iodide, 0.08 gm.; sodium fluoride, 1 gm.; aluminum potassium sulfate, 0.17 gm.; copper sulfate, 0.19 gm.; and $MnSO_4$ increased from 0.35 gm. to 0.5 gm. per cent (of total salt).

form of wheat germ oil (Sharpe and Dohme). Vitamin C was omitted because these animals can synthesize enough ascorbic acid to avoid scurvy.[23]

ACUTE VITAMIN K DEFICIENCY

Chicks

Clinical Observations

Two-day-old white Leghorn chicks were maintained on a normal diet and observed in laboratory condition for 2 or 3 days. Apparently normal, selected chicks were then put on Dam-Schönheyder's modified diet, as described above.

The first signs of deficiency appeared in approximately 7 to 14 days after the beginning of the experiment.* Usually the hemorrhagic manifestations were preceded by some changes in the general appearance and behavior of the chicks. Some appeared hyperactive and somewhat restless in that they were irritable and had a tendency to scratch themselves, particularly around the neck, wings, thorax, tail, and abdomen. Close observation frequently revealed congestion of the superficial blood vessels, particularly around the knee joints and the ventral surface of the wings and toes. At the same time some of the chicks limped and had a tendency to sit down after taking only a few steps. They seemed fatigued and unable to maintain the body in an erect position. At times they tried to support themselves by leaning against the walls of the cage. In eating they often sat down. Concomitantly, more or less pronounced swelling of the knee joints with marked dilatation of the blood vessels (as revealed by transillumination) was frequently noticed. Fighting was quite often observed, after which bleeding sometimes persisted for several hours. At that stage, changes in the general aspect of the feathers and loss of feathers were noticed (Fig. 1).

Approximately after 8 to 15 days, more frequent and more marked hemorrhages were noticed. Their form, size, and diffusion varied from chick to chick. Although they seemed to appear spontaneously, we also had the impression that the hemorrhages easily followed very small and almost insignificant injuries. These were frequently aggravated by self-scratching or "picking." The hemorrhagic manifestations were more common around the roots of the feathers over the neck, breast, wings, thighs, and on the legs, toes, and toenails. Generally, bleeding was more pronounced in those regions which were more exposed to mechanical irritation or trauma. At times, the hemorrhages were localized in the legs, joints (Fig. 2), or wings (Fig. 3). At others, they were markedly diffuse and persisted until death. They were present more

* We gained the impression that there was a seasonal influence in relation to the appearance and intensity of the hemorrhagic manifestations. They appeared more promptly and were more marked in February, March, and April.

frequently in the superficial tissues although observable also in the deeper tissues and the viscera. In many instances the stools appeared stained with blood. The benzidine test as well as the Meyer reaction for blood was intensively positive.

During the hemorrhagic stage some of the chicks disclosed hypermetria or dysmetria, noticeable in their gait, anterolateral pulsion or retropulsion, poor equilibrium in the erect position, and at times increased muscular tonus in the legs ("support reaction"). One chick presented marked unilateral exophthalmos due to a retrobulbar hematoma found at autopsy. Some gradually became unable to walk or to maintain an erect position. On some occasions chicks were seen lying on their sides with hyperextended extremities. In a few animals involuntary movements and abnormal posture of the head and neck were observed.

At this stage of the experiment death of some birds occurred almost suddenly. With others, death was preceded by an agonal stage, the duration of which varied from a few hours to a day. During this period irregular clonic-tonic or choreo-athetosic movements were noticed occasionally. In a few instances marked opisthotonus was present, somewhat similar to the posture described by Pappenheimer and associates [58] in chicks suffering from encephalomalacia.

The majority of chicks were sacrificed by ether, while some were autopsied immediately after spontaneous death. Tissues were fixed in 10 per cent alcohol, neutral formalin, formol-bromide, Zenker's, and Bouin's solutions. For microscopic investigation the following histologic methods were used: hematoxylin and eosin, Masson's trichrome stain, Weigert's stain for elastic tissue, and van Gieson's method for connective tissue. For the nervous system, Nissl's stain for nerve cells, Hortega's silver carbonate impregnation for microglia and oligodendroglia, Cajal's gold sublimate for astroglia, and Roizin's[57a] combined method for myelin sheaths and lipid products of disintegration were used. For the capillary network, Pickworth's [57b] and Eros' [57c] methods were applied.

Autopsy Findings

Macroscopically, there was marked congestion, and small and large hemorrhages were noticed in the subcutaneous tissue, muscles (Figs. 4, 5, and 6), knee and shoulder joints, periosteum and bone, retro-ocular regions, lungs, gizzards (Fig. 7), intestines, liver (Fig. 8), kidney (Fig. 9), meninges, and brain.

The microscopic studies confirmed the occurrence of hemorrhagic manifestations in the skin and muscles (Fig. 10), joints and bones, esophagus, gizzard (Fig. 11), intestine (Fig. 12), liver (Fig. 13), sub-

capsular and parenchymatous regions of the kidneys (Figs. 14 and 15), heart (Fig. 16), lungs (Fig. 17), spleen, gonads, thymus, meninges, choroid plexus and ventricles, brain stem, cerebellum, and spinal cord.

In many instances, the hemorrhages were within the confines of the perivascular spaces forming an actual extramural hematoma; in others they were free in the parenchymatous tissue. The hemorrhages appeared as round, oval, or irregular, and varied greatly in size, from small capillary or precapillary areas to those that could be detected with the naked eye. Frequently they enveloped the blood vessels in "cuff-like" fashion. In some sections, no blood vessels could be discerned within the hemorrhagic area; in other areas, several blood vessels were included in it (Fig. 18). In some instances several hemorrhagic foci appeared, separated only by scanty and disorganized parenchyma (Fig. 19).

Some hemorrhages were so recent that relatively little degenerative change had occurred in the blood elements or in the involved tissues. In other areas the tissue of the involved organs was infiltrated with blood elements in various stages of disintegration. Numerous large and smaller mononuclear phagocytes containing brownish or yellowish amorphous granules or pigment were often found in these areas (Fig. 20). Some of these granular products gave a positive Perles' reaction for iron. In the brain tissue, phagocytic elements originating from microglial cells were often encountered.

Histologic studies revealed also various changes of the blood vessels. A majority of the arteries and veins were dilated; in some cases the capillaries appeared numerous and hyperemic (Fig. 21). Frequently, globular, ampullar, or irregular dilatations of the blood vessels and capillaries were noticed (Figs. 22, 23, and 24). In some instances the intimal cells of the arteries were swollen and pale, with the remainder of the wall edematous or thickened (Fig. 25). Both homogenization of the walls of vessels and discontinuity of some of the layers or rupture were noticed (Fig. 26).

The veins frequently appeared markedly distended, their walls irregular and often thinned. The intimal cells were round or ovoidal and at times pale or colorless, the muscle layer hardly discernible, and the adventitia poorly demarcated (Fig. 27). Although the vascular changes were generally seen in areas involved by hemorrhages, they were also encountered in the neighboring or even in far distant regions. Frequently, modifications of the content of the blood vessels were evidenced by a central conglomeration of the erythrocytes with peripheral displacement of the leukocytes (Fig. 28) with some diapedesis and perivascular edema.

Rats

In the first group of experiments, 20 albino rats, weighing from 120 to 250 gm., were maintained on the same vitamin-deficient diet as used for chicks, for a period of approximately 10 months. Grossly, no definite hemorrhages were observed with the exception that some rats presented somewhat increased bleeding time and prolonged clotting time.

In a second group of 20 young rats put on the same vitamin K-deficient diet at the time of weaning (average weight, 38 to 45 gm.) from 7 to 12 days after the beginning of the experiment, hemorrhagic manifestations were noticed in the nails (Figs. 29 and 30) or around the toenails and the skin of the toes.

A third group of 15 adult, pregnant rats were put on the same vitamin K-deficient diet. Vitamin E (alpha-tocopherol, Merck) was, however, administered by injection to secure a normal pregnancy (as suggested by Pappenheimer *et al.*).[58] The offspring of these rats presented spontaneous hemorrhagic manifestations immediately or a few days after birth, which were identical to those described in the chicks, although somewhat less frequent and less diffuse. With the addition of from 5 to 10 per cent mineral oil [25] to the deficient diet, the bleeding appeared more marked and more diffuse. In these rats the hemorrhages were observed more frequently in those regions of the body exposed to mechanical irritation or traumatization, especially the tail, toes, nails, feet, legs (Figs. 31 and 32), abdomen, thorax, and occasionally the face; also, subcutaneous tissue and muscles (Fig. 33), knee joints, subarachnoid space (Fig. 34), retro-ocular region (Fig. 35), pelvic region (Fig. 36), some of the abdominal and thoracic viscera, brain, and cerebral ventricles (Fig. 37).

From a clinical point of view the rats did not exhibit the dysmetria, loss of equilibrium, and fatigability described in the chicks. However, some of the animals revealed limping or slight spastic paraparesis, which occurred mostly in the very young and was transient in character. One of the rats presented a marked bilateral and asymmetric exophthalmos which at autopsy was related to a retro-ocular hematoma. Another young rat developed generalized tonic-clonic convulsions a few hours before death. Autopsy revealed the presence of massive intracerebral and intraventricular hemorrhages.

Histologic studies of the various tissues and organs revealed changes of the same type and character as those described in the chicks, although generally less numerous and involving mostly the external tissues (such as skin, subcutaneous and muscular tissues).

SUBCHRONIC AND CHRONIC VITAMIN K DEFICIENCY

The chicks and rats used for this investigation were maintained on the basic vitamin K-deficient diet until they revealed the first sure signs of vitamin K deficiency, *i.e.*, spontaneous hemorrhages and prolonged bleeding. At this point the animals were fed daily either the synthetic vitamin K (Proklot, Lilly *) alone or with addition of biliary salts (Bilron, Lilly *) until the initial external hemorrhagic manifestations completely disappeared. Some of these animals did not benefit from the addition of vitamin K (combined or not with Bilron), although the majority improved considerably or recovered completely. When recovery occurred (from 4 to 7 days), the animals were reinstated on the original vitamin K-deficient diet. A few animals spontaneously survived the acute episode without the addition of the synthetic vitamin or biliary salts. These animals were also included in this group.

Chicks

The chicks treated as above, *i.e.*, allowed to recover from the first acute episode, and then reinstated on the original vitamin K-deficient diet, survived for months up to 1½ years, when they were sacrificed for experimental needs. The majority developed well and, as far as curves of growth are concerned, showed no marked difference from a control group maintained on the synthetic vitamin-deficient diet to which vitamin K (Proklot) was added. However, some of the chicks reaching the adult age presented various alterations of the knee joints and bones which will be discussed in detail later.

A large number disclosed changes in the general aspect of the feathers (roughness and fragmentation). Also, loss of feathers occurred, usually in the regions more exposed to mechanical irritation (the anteroventral region of the thorax, and the abdominal regions), around the tail, and less frequently and in a lesser degree, on the legs and dorsal region of the body.

The hemorrhagic manifestations were much less frequently observed than in the acute phase of the experiment and they were more localized. When present, they generally appeared at the roots of the feathers or as small or larger hematomas located mostly in the subcutaneous tissue (Fig. 38). At times small and large hematomas were found at autopsy in the deeper tissues (Fig. 39), abdominal cavity, and various viscera (Fig. 40).

Small hematomas were reabsorbed or organized, but occasionally

* These products were kindly supplied by Eli Lilly Co. to whom we wish to express our thanks.

they ruptured by traumatization or spontaneously, leading in some instances to the death of the animal (Fig. 41).

In post-mortem material three common alterations involving the different tissues and organs in various degrees were noticed: hemorrhages, vascular alterations, and reactive or degenerative changes directly or indirectly related to the other two.

The hemorrhages were either of recent character or older. The more recent were very similar to those described in the acute stage of vitamin K deficiency, although less frequent and less diffuse. The older ones could be divided into two types: hematomas more or less organized (Fig. 42), variable in size and in form; and more or less free, massive hemorrhages. Histologically, the latter appeared generally as hemorrhagic masses showing varying degrees of disintegration. In the majority the blood filled the interstitial spaces or replaced the destroyed structure of the organ. In the lung, for instance (Fig. 43), many of the alveoli were stuffed with blood and their outlines were no longer visible, owing to necrosis of the alveolar walls. The cellular details were lost and only shadowy network of the tissue remained to identify lung. Here and there large amounts of amorphous, golden brown or granular pigment, most of it intracellular within large mononuclear phagocytic cells, was observed. Some of these phagocytic elements containing iron pigment could be compared to the "dust cells" of anthracosis. In certain areas, extracellular pigment was also noticed where red cells had disintegrated in the interstitial tissue. Similar changes were noticed in the kidney (Fig. 44), spleen, brain (Fig. 45), and other tissues.

The blood vessels and the perivascular spaces of the various viscera and tissues, mostly when surrounded by blood or blood pigments, revealed differing pathologic changes. In some, the perivascular spaces appeared enlarged and contained, at times, various amounts of fatty products of degeneration (Fig. 46). In others, the surrounding tissue elements were also undergoing marked fatty degeneration (Fig. 47). In the central nervous tissue, particularly in the white matter of the brain, some of the blood vessels were surrounded by gitter cells containing various amounts of products of myelin disintegration besides hematic pigments, as well as progressive, reactive, and degenerative changes of the glial elements. Here and there perivascular demyelination also was observed (Fig. 48).

The vascular walls themselves were variously involved. In some the intimal cells were swollen, pale, and occasionally appeared thickened and hyperplastic. Even stratification of the endothelial cells was present (Fig. 49). The media, at times, showed degeneration of muscle fibers or increase in connective tissues, demonstrated by van Gieson's

method for connective tissue. Weigert's method for elastic tissue revealed some discontinuity and disintegration of the elastica and only occasional hyperplastic proliferation of the elastic fibers. In some instances the adventitia of the blood vessels was found thickened and marked proliferation of the connective tissue was noticed. Occasionally, in certain arteries, thickening of the entire walls was observed (Fig. 50). However, stasis and pre-stasis, as expressed by central conglomeration of erythrocytes, peripheral displacement and diapedesis of the white blood cells, as well as some vascular changes (edema and occasional rupture of the blood vessel walls) described in the acute phase of vitamin K deficiency, were also encountered at times in the subchronic and chronic group of vitamin K deficiency.

Parenchymatous histologic changes were present in the lungs, heart, liver, spleen, kidneys, central nervous system, muscles, testicles, thymus, and skin. These structural alterations, the description of which follows, were more or less closely related to the vascular pattern or to the hemorrhagic condition.

These parenchymatous structural changes appeared mostly as scattered areas of variable size and shape, undergoing degeneration and surrounded by more or less normal structures. These changes varied from slight cloudy degeneration to severe degeneration and even complete disintegration or disorganization of the basic histologic structures (Fig. 51). Here and there foci of necrosis were detected. Débris of the morphologic elements of the blood and hematic pigments were observed quite frequently in the same areas. Generally, disintegrated extravasated blood was free in the tissues but in some instances was in mononuclear phagocytes and macrophages.

In the liver, at times, striking distention of the sinusoids, especially in the center of the lobule, was seen. The sinusoids were then bordered by markedly compressed liver cords. For the most part the liver parenchyma showed degenerative changes only occasionally. However, in the tissues surrounding the blood vessels fat-staining methods revealed fatty products of degeneration which were frequently observed also in the blood vessel walls themselves.

In the nervous tissue, in addition to the already described morphologic alterations, occasional areas of demyelination, mainly surrounding blood vessels, were observed. There the combined method for myelin sheaths and lipids disclosed also the presence of lipidic products of degeneration (Fig. 52). In the brain and cerebellum scattered cortical areas disclosed nerve cell degeneration and acellular areas of varied dimensions. In the cerebellum small areas were found in which most of

the Purkinje and granular cells had disappeared with only remnants of cells in the acellular areas (Figs. 53 and 54).

In all affected viscera and tissues, scattered areas revealing more or less intense reparative processes often were observed. In these areas it was almost impossible to recognize the original structure of the organ replaced by proliferated connective tissue, leading at times to fibrosis (Figs. 55, 56, and 57) and scar-like formation or, in the brain, to glial hypertrophy and hyperplasia (Figs. 58 and 59).

Rats

Rats were treated in the same manner as the chicks of the corresponding subchronic and chronic group. Generally speaking, one may say that in chronic vitamin K deficiency there were some slight differences between the results in the two groups: (a) In the chronic as well as in the acute stages, hemorrhages were more frequent in chicks than in rats; (b) In younger rats in the chronic state of deficiency, although hemorrhages were more frequent than in adults, this difference was less pronounced than in the chicks under the same experimental conditions. Furthermore, in rats of the second generation, the offspring of those maintained on a vitamin K-deficient diet to which vitamin E (alpha-tocopherol) was added during pregnancy, the frequency and the intensity of hemorrhages were greater than in rats born of normal parents; (c) Spontaneous recovery (from the acute deficiency) was more frequently noticed in the rats particularly after the weaning age. (d) Reabsorption of small hematomas was more frequent in the more mature or adult rats than in the chicks, although some localized and organized hematomas were noticeable as much as 14 months later (Fig. 60).

The histopathologic findings in this group of rats were similar to those described in the chicks in the subchronic and chronic stage with the exceptions that: (1) The hemorrhagic manifestations and secondary structural changes of the involved tissues were found mostly in the superficial tissues (particularly skin, subcutaneous tissue, and muscles); (2) These morphologic changes were much less frequently encountered than in the corresponding chick group.

One rat (second generation) kept on the vitamin K-deficient diet for 340 days gradually developed an apparent genito-lipodystrophy somewhat similar to Fröhlich's type. The animal weighed 480 gm., as compared with 350 gm. for a normal control. At autopsy a small organized hematoma in the retrosellar region of the skull was found (Fig. 61). Through compression it produced a marked depression at the

base of the brain involving the retrochiasmatic portion of the hypothalamus, the mammillary bodies, the fossa interpeduncularis, and a small part of the anterior portion of the pons (Fig. 62).

Some incidental histologic findings in chicks seemed to us the expression of complicating factors rather than of pure vitamin K deficiency.

(a) Marked proliferation of capillaries in the brain was observed in certain cases (Fig. 63a) and recalled somewhat similar vascular changes noted by us [26] in the cerebral cortex of cats in experimental inanition, and by Alexander [27] and Zimmerman and Burack [28] in selected areas in the central nervous system in vitamin B_1 deficiency.

(b) Gizzard erosions were found mostly in chicks in the acute stage and in a very few chicks in the subchronic stage of vitamin K deficiency.

(c) Small areas of encephalomalacia (Fig. 63b) were occasionally encountered. These areas were scattered in the cerebrum and cerebellum and consisted, especially in the latter, of degenerative changes of the parenchyma. Nerve cells, myelin sheaths, nerve fibers, and glial elements exhibited signs of advanced disintegration up to complete demyelination. Surrounding these areas reactive progressive changes of the glial elements were found. Similar areas of encephalomalacia were described in chicks by Pappenheimer and Goettsch [29] and Wolf and Pappenheimer, [30] and were considered by them to be the expression of vitamin E deficiency.

(d) On a few occasions, granulomatous formations with giant cells were noticed in the lungs. Some were fibrotic and calcified. Occasionally, marked calcification of the alveolar tissue independent of the granulomatous reaction also was observed.

(e) Another finding of interest from the standpoint of symptomatology in chicks was the occurrence in some of them of chondro-osteodystrophies in the subchronic and chronic group. This condition usually developed gradually. At first the chick experienced some difficulty in walking consisting chiefly in fatigue of the extremities and occasional limping. A few days later slight swelling of the knee joints was noticed which increased gradually in the subsequent days. Although this phenomenon more frequently involved only one side (Figs. 64 and 65), now and then bilateral swellings were observed. The chicks would then remain generally in a sitting position (even when eating or drinking). When stimulated to walk, they would support their bodies on the normal side, maintaining the affected extremity mostly in a flexed position. With the progress of time the involved extremity was less and less used while the flexor contraction would become more pro-

nounced and permanent. When the involvement was bilateral the chick became unable to walk (Fig. 66). At this stage slipping of the tendon from its proper position was frequently observed, as illustrated in anatomic preparations (Figs. 67, 68, and 69). In addition hemorrhages were observed in some of the affected joints and periarticular tissues. Roentgenologic examinations and gross and histologic studies revealed edema of the periarticular and articular tissues in addition to displacement, deformity, or dystrophy of various degrees involving the cartilages or the bony tissue, or both (Figs. 70, 71, and 72).

In the early stages of our investigation we felt that this condition was related to vitamin K deficiency. Later on an analysis of the diet pointed to a deficiency in manganese and that the condition was related to the so-called perosis or slipped tendon disease which was considered by Wilgus, Norris, and Heuser [31, 32] and Inskso, Lyons, and Martin [33] as due to dietary manganese deficiency. To establish which part of the symptomatology was related to such deficiency, 20 white Leghorn chicks were put on the same vitamin K-deficient diet as used in the previous experiments with the exception that the quantity of $MnSO_4$ of the salt mixture was increased from 0.35 to 0.5 gm. per cent (of total salt mixture). In this group we did not observe the typical slipped tendon condition. However, at times swelling of the knee joints, occasionally associated with abnormality of the posture or disturbed walking and some chondro-osteodystrophic changes, were still noticed although milder in intensity and much less frequent. Some of these alterations were considered by us as having definite relationship to the presence of hemorrhages or hematomas within the articulations (Figs. 73, 74, and 75) and in the bony tissue itself. This last occurrence in one case resulted in a fracture of bone (Fig. 76).

Discussion and Comments

The opinion expressed in our previous report [22] on the histopathology of the central nervous tissue, concerning the appearance, frequency, and intensity of vitamin K-hemorrhagic diathesis, may be extended also to the other tissues and organs; *i.e.*, that in addition to the biochemical and physiopathologic effect proper to vitamin K deficiency, two main factors, age of the animals and action of mechanical irritation or external trauma, play an important rôle. In relation to the "age factor" we observed that the hemorrhagic diathesis was more marked and frequent in young chicks (7 to 12 days old) and in young rats (3 to 20 days old) than in adult animals which eventually become better adjusted to vitamin K deficiency.

The gross and microscopic changes noticed in the experimental

animals have a certain similarity to that which is known in human pathology as "bleeding disease of the newborn infants" or "morbus hemorrhagicus neonatorum." In fact, a large number of clinical investigations corroborate this similarity. In 1937, Brinkhous, Smith, and Warner [34] found that prothrombin in a bleeding newborn baby was exceedingly low and stated that the cause of the bleeding was hypoprothrombinemia. Later on Quick and Grossman [35] found that in all newborn infants there is a definite phase of physiologic hypoprothrombinemia from the second to the fifth day of life. Meanwhile Salomonsen [36] and Grossman [37] found that cerebral bleeding revealed itself precisely between the second and the third day of life. Further, Dam, Tage-Hansen, and Plum,[38] Nygaard,[39] and Quick [40] concluded that the physiologic hypoprothrombinemia is due to a dietary lack of vitamin K in the first few days of life and that subsequent establishment of the bacterial flora in the baby's intestines initiated the synthesis of vitamin K or similar antihemorrhagic substances which became an important factor in the spontaneous restoration of the normal prothrombin level.

As far as the action of mechanical irritation or external trauma is concerned, we observed particularly in both young chicks and young rats that the lesions were more pronounced in those regions of the body that were more exposed to mechanical irritation or external trauma. External hemorrhages appeared more frequently and were more pronounced when many animals were kept in the same cage making them more subject to fighting and to trauma. While from 60 to 70 per cent of the animals (mostly young, and particularly chicks) exhibited a cutaneous hemorrhagic diathesis, only 20 to 26 per cent presented visceral hemorrhages and even less (3 to 5 per cent) presented hemorrhages in the central nervous tissue. These figures seem to be related to the better protection of the viscera and especially the central nervous tissues against mechanical irritation or external trauma. In bleeding disease of the newborn human, it has also been found that trauma, and particularly the application of forceps, was a precipitating factor. Besides, Snedeker [41] reported that during the periods of physiologic hypoprothrombinemia minor operations such as circumcision or cutting of the frenulum of the tongue could be followed by unremitting bleeding which occasionally led to exsanguination.

Cattle and other animals, particularly ruminants, fed spoiled sweet clover hay develop a hemorrhagic diathesis characterized by prolonged clotting time and hypoprothrombinemia. [42] These animals often carry the disease in a latent form, but a slight trauma may bring about severe hemorrhages which may frequently terminate fatally (Rod-

erick [43]). However, it was demonstrated that hypoprothrombinemia in these animals is not due to vitamin K deficiency but to the reaction of a toxic substance, dicoumarin. [44-46]

Although age and trauma play a precipitating rôle in the causation of hemorrhages, the genesis of the hemorrhagic diathesis in the course of vitamin K deficiency lies fundamentally in the general or local biochemical and pathophysiologic changes which, according to the majority of authors, result from the reduction of prothrombin in the blood of both man and experimental animals. Through studies of blood clotting time it has been established that hypoprothrombinemia may be due either to the lack or insufficiency of vitamin K in the diet, or to its disturbed metabolism related to hepatic dysfunction or disturbed internal absorption. That the absence of vitamin K from the diet, in our experimental group of animals, is responsible for the genesis of hemorrhages was established not only by the fact that hemorrhages appeared only in animals fed with the Dam-Schönheyder modified diet, but also by the cessation of visible hemorrhages and recovery of animals following administration of synthetic vitamin K (Proklot, Lilly).

It has been reported by Elliott and his co-workers [25] that the addition of mineral oil to the diet causes hypoprothrombinemia by preventing absorption of vitamin K or by producing injuries to the liver. In our group of animals in which mineral oil was added to the diet the hemorrhagic diathesis appeared sooner and was more severe, thus supporting the hypothesis that mineral oil adds to the deficiency either by interfering with the assimilation of whatever trace of vitamin K may be present or inhibiting the intestinal fermentation (through which synthesis of vitamin K or similar anti-hemorrhagic substances may originate).

Our experimental data find their counterpart in human pathology. Recently it has been established that the tendency toward bleeding in intestinal disorders,[47-49] obstructive jaundice,[50] biliary fistula,[51] and liver diseases [52, 53] is related to an altered process of absorption or to defective metabolism of the anti-hemorrhagic factor rather than to a primary dietary deficiency of vitamin K. Because of this supposed relation between liver function and the metabolism of vitamin K, it has been suggested that bile salts be administered in addition to vitamin K to prevent the symptoms caused by such a deficiency. Guided by this suggestion, we have made use of biliary salts in our experimental work in order to secure more prompt action of vitamin K in preventing the early hemorrhagic manifestations in those animals in which recovery was attempted before resumption of the subchronic experiments.

In addition to the action of precipitating factors such as age and

trauma, we feel that our histologic studies furnish some additional information concerning the physiopathologic mechanism of the hemorrhage itself. It is known that retardation of the blood current, through pre-stasis and stasis, is considered a factor predisposing to diapedesis of hemal elements. In addition, Landis [54] and Baron and Chambers [55] stated that stasis and typical concentration of red cells in the central part of blood vessels, as described by Krogh,[56] can result also from local mechanical injuries to blood vessel walls themselves without changes in diameter. Pickworth [57b] believed that central concentration of red blood cells and peripheral displacement of the white cells in the blood vessels are in relation to hydrodynamic changes in the circulation of the blood. Figures 27 and 28 illustrate the abnormal disposition of the white and red blood cells as well as a stage of their migration. We feel that vasodilatation and the marked congestion of the blood vessels occurring in the early stage of deficiency may play a part in the production of the hemorrhage.

Possibly the lack or deficiency of prothrombin may determine some disturbance of the hydrodynamics of the blood in addition to physicochemical or morphologic changes of the vessel walls which would facilitate diapedesis.

We would like to discuss briefly certain previously listed pathologic findings in chicks on the vitamin K-deficient diet, which require further investigation.

(a) In some areas of the brain marked proliferation of capillaries was noticed in a few chicks in the subchronic and chronic stages of vitamin K deficiency. This histopathologic finding recalled somewhat similar vascular changes described by us in the brain and brain stem of cats in experimental inanition [26] and by Alexander (pigeons) [27] and Zimmerman and Burack (dogs) [28] in the central nervous system in vitamin B deficiency. This may be related to other factors than simple vitamin K deficiency, such as general malnutrition or disturbed assimilation of vitamins B and B-complex.

(b) The presence of "gizzard erosions" was noticed in some of the chicks with hemorrhagic diathesis of the gizzard itself. On the other hand, many chicks with no appreciable hemorrhages in the gizzard revealed in the latter very severe erosions. In this regard our findings are in full agreement with those of Dam and Schönheyder,[20] Holst and Halbrook,[19] and Almquist and Stokstad.[4] We also agree with their opinion that gizzard erosions are not a characteristic symptom of vitamin K deficiency and that they may be initiated, to some extent, by traumatic stimuli.

(c) In a few chicks only, scattered small areas of encephalomalacia

in the brain and cerebellum were found. These areas are somewhat similar to those described in chicks by Pappenheimer and Goettsch [29] and later by Wolf and Pappenheimer [30] and considered by them and other workers as the expression of vitamin E deficiency.[58-60] We feel that in our animals there was no deficiency in vitamin E. Nevertheless, deficiency of vitamin E may originate in disturbed metabolism of other vitamins or nutritional factors. Such inter-relationship, if it exists, must be left for further investigation; especially in connection with the presence in the diet of various excessive fatty acids (Dam [60]).

(d) In the lungs of a few chicks in the subchronic and chronic stages of vitamin K deficiency granulomatous formations with giant cells were found. Some of them were fibrotic and calcified. No direct relationship with hemorrhages was detected. It seems that these histopathologic changes might be considered as a result of a complicating factor, such as secondary infections, rather than the direct effect of the vitamin K deficiency.

(e) The rôle of manganese in the prevention of perosis or slipped tendon disease was indicated by the observations of Wilgus *et al.*,[31,32] Gallup and Norris,[61] and Inskso *et al.*[33] Later on, another group of authors (Hogan and Richardson,[62] Jukes,[63,64] Hegsted, Mills, Elvenhjem, and Hart,[65] reported that perosis, which occurred in spite of adequate dietary manganese, was prevented by constituents of an alcoholic extract of liver (Hogan *et al.*[62]), choline (Jukes,[63,64] Jukes and Welch [66]) and possibly by certain unidentified factors (Jukes,[64] Hogan *et al.*[62]). In our experiments we observed that the addition of a larger quantity of manganese to the vitamin K-deficient diet prevented only some of the changes related to slipped tendon disease or perosis, thus leaving the impression that a portion of this chondrodystrophy was related to the hemorrhages (Figs. 74, 75, and 76). On this point our findings are somewhat similar to the orthopedic findings of König [67] in 98 cases of hemophilia. He described: (1) "hemarthrosis" or the stage of the first hemorrhages into the joints; (2) "panarthritis" or the stage of inflammatory reaction with changes in the bones and cartilages; (3) "regression" or the stage of permanent joint changes with erosion and contractures (Figs. 75 and 77).

Once hemorrhages have occurred in the joints, they exercise harmful effects, partially through a mechanical factor (pressure atrophy, etc.) and partially through inflammatory secondary changes of the synovial membrane, an opinion already expressed by König[67] and Fonio.[68]

We believe that trauma plays a very important rôle as a precipitating and aggravating factor in the pathogenesis of some of the chondrodystrophic changes. This is illustrated by the fact that the joints in-

volved are generally the knees (Figs. 65, 66, 67, 74, and 75), which the chicks used as the main support whenever unable to walk normally. Such continuous mechanical irritation leads to various secondary alterations and deformities in the joints and bones (Figs. 71, 72, and 73). Our observations agree with those of Fonio,[68] who reported that in cases of hemophilia the joints commonly involved are those most frequently exposed to trauma; namely the knee, the elbow, and the ankle.

Summary

White Leghorn chicks and albino rats (first and second generation) in acute, subchronic, and chronic vitamin K deficiency revealed various pathologic findings:

1. Hemorrhagic diathesis. This condition was characterized by hemorrhages in the skin, subcutaneous tissue, joints, muscles, lungs, heart, gizzard, liver, kidneys, spleen, bones, thymus, gonads, brain, spinal cord, meninges, choroid plexus, subarachnoid spaces, and cerebral tissue. Frequently the hemorrhages were within the confines of the perivascular spaces and enveloped the blood vessels in "cuff-like" fashion; at other times they were free in the parenchyma. The hemorrhages appeared round, oval, or irregular and varied greatly in size, from small capillary or precapillary to large hemorrhages easily detected with the naked eye. Often the hemorrhages appeared as scattered foci, some were separated only by scanty and disorganized interstitial or parenchymatous tissue, and others showed confluence of various foci. The hemorrhages were recent or older, depending upon the time of observation. At times they were more or less organized hematomas, or large phagocytic elements containing hematic granular pigment represented the only trace of previous hemorrhages. Recent hemorrhages were noticed more frequently in the acute stage, whereas hematomas and phagocytes containing hematic pigments were encountered more frequently in the subchronic and chronic stage.

2. Blood vessel changes. Frequently the blood vessels (veins and arteries) appeared markedly and irregularly dilated and congested. Here and there their walls appeared variously involved, from simple swelling and mild degeneration to complete rupture. These vascular changes were observed more frequently near or within the hemorrhagic areas. In some sections no blood vessels could be discerned within the hemorrhagic infiltrated tissue. Hypertrophy and hyperplasia of the blood vessel walls were also encountered. The latter was more often observed in the subchronic and chronic stage of deficiency.

3. Hydrodynamic disorders of the circulation as expressed by prestasic and stasic conditions were noted in the acute and subchronic stages, more often in the former.

4. Degenerative and reparative structural changes of different tissues and viscera, mostly secondary to the hemorrhagic conditions, were observed in both subchronic and chronic stages.

5. Proliferation of capillaries in certain areas of the brain, gizzard erosions, areas of encephalomalacia, granulomatous formations, and perosis or slipped tendon disease were noticed only in chicks. The second and third were observed more frequently in the acute and subchronic stage, the other two mostly in the subchronic and chronic stage. These changes were considered the expression of complicating factors. Determination of their relationship to vitamin K deficiency will depend on further experimentation.

The following factors played a rôle in experimental hemorrhagic diathesis in vitamin K deficiency:

1. Altered physicochemical properties of the blood, presumably hypoprothrombinemia, which may interfere with the proper hydrodynamics of the circulation of the blood and result in vasodilatation, stasis, diapedesis, and structural damage of the blood vessel walls.

2. Age of the animals, since hemorrhages were more severe and more prompt to appear in newborn or young animals.

3. Traumatic factors seem to play a definite precipitating rôle.

4. Dysfunction in the absorption (especially when mineral oil is added to the diet) and metabolism of vitamin K and possible hepatic dysfunction.

Our findings are similar to those in the bleeding disease of the newborn infant (morbus hemorrhagicus neonatorum), a condition which is also aggravated by trauma.

Gizzard erosions and encephalomalacia of chicks were not considered characteristic of the vitamin K-deficient diet, presumably being related to other nutritional factors still to be investigated.

The chondrodystrophies observed mostly in chicks in the chronic stage seemed to be partly related to perosis or slipped tendon disease and partly to hemarthrosis. The latter was considered as the expression of the hemorrhagic diathesis. Trauma seemed also to be an important factor in determining and aggravating the chondrodystrophies.

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DESCRIPTION OF PLATES

PLATE 213

FIG. 1. Changes in the general aspect of the feathers and falling of feathers in chicks in acute vitamin K deficiency.

FIG. 2. Hemorrhages in a knee joint of a chick in acute vitamin K deficiency.

FIG. 3. Hemorrhagic suffusion in the subcutaneous tissue of a wing in a chick in acute vitamin K deficiency.

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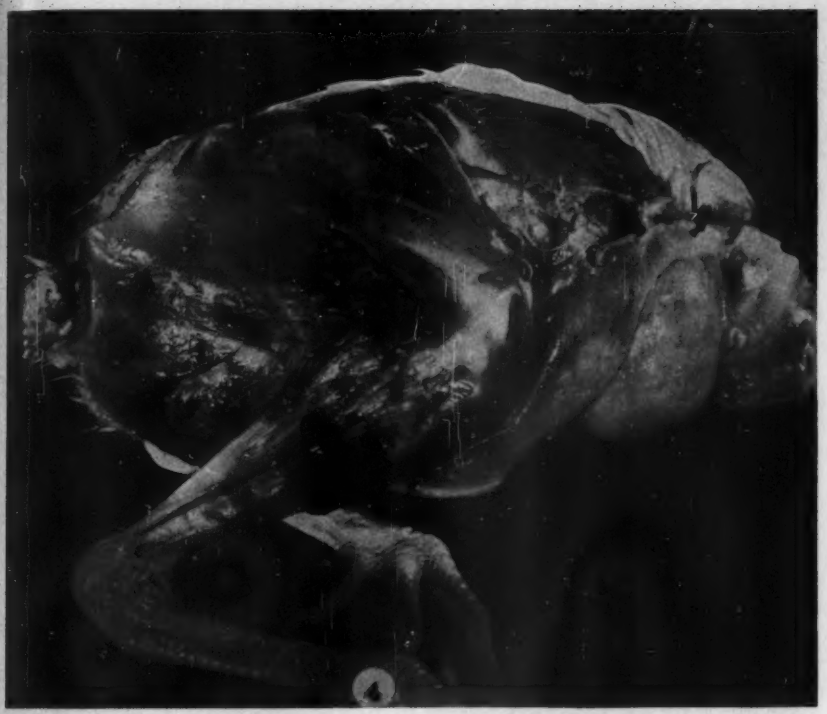
PLATE 214

FIG. 4. Subcutaneous hemorrhagic blood clots, particularly pronounced in the antero-external surface of the leg of a chick in the acute stage of vitamin K deficiency.

FIG. 5. Diffuse infiltrating hemorrhages in the muscles of the leg of a chick sacrificed in the acute stage of vitamin K deficiency.

FIG. 6. Severe infiltrating hemorrhage in the pectoral muscle of a dead chick in the acute stage of vitamin K deficiency.



Ferraro and Roizin Diathesis by Deficiency in Vitamin K

PLATE 215

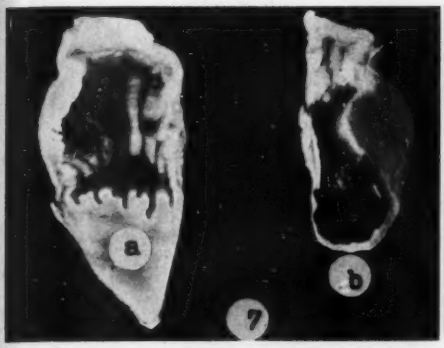
FIG. 7. Erosions (a) and hemorrhages (b) of various degrees and extension in gizzards in the acute stage of vitamin K deficiency.

FIG. 8. Infiltrating hemorrhage in the liver of a chick sacrificed in the acute stage of vitamin K deficiency.

FIG. 9. Infiltrating hemorrhages in the kidney of a chick sacrificed in the acute stage of vitamin K deficiency.

FIG. 10. Histologic appearance of infiltrating hemorrhages of the skin, subcutaneous tissue, and subjacent muscles of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 34.

FIG. 11. Histologic appearance of hemorrhages involving the superficial layers of the gizzard in a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 20.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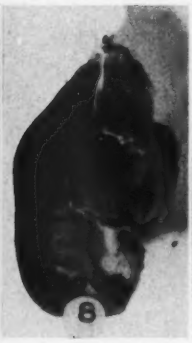
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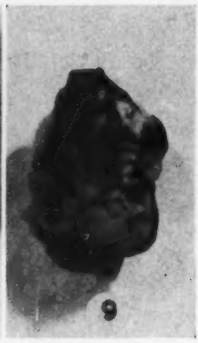
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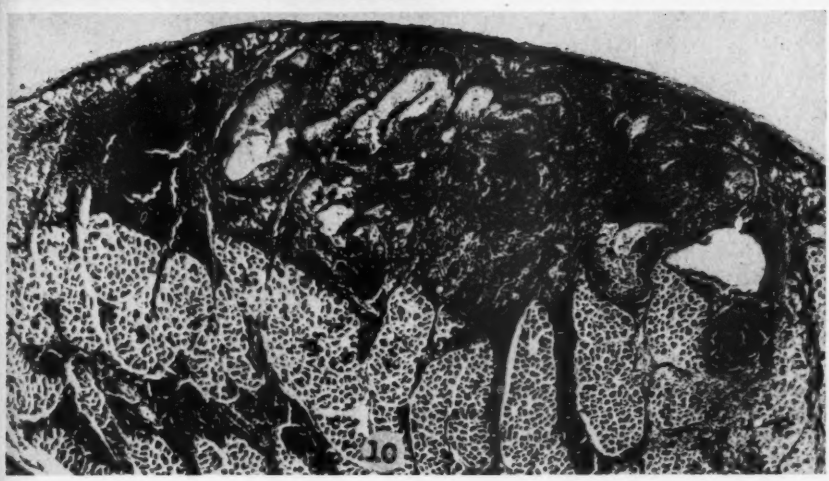
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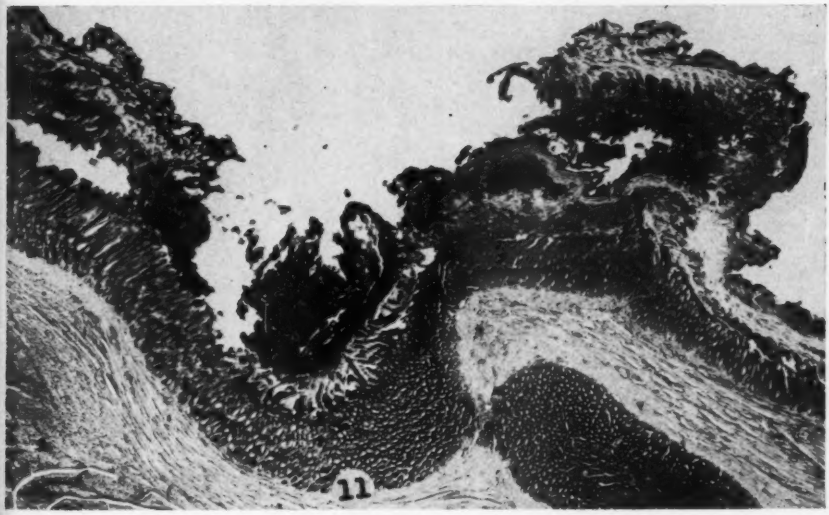
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PLATE 216

FIG. 12. Hemorrhages involving mucosa, submucosa, and muscular layers of the small intestine of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 100.

FIG. 13. Hemorrhages of varying size and form in the liver of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 200.

FIG. 14. Histologic appearance of a massive subcapsular hemorrhage in the kidney of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 52.

FIG. 15. Hemorrhages mainly involving the tubular structures of the kidney of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 100.

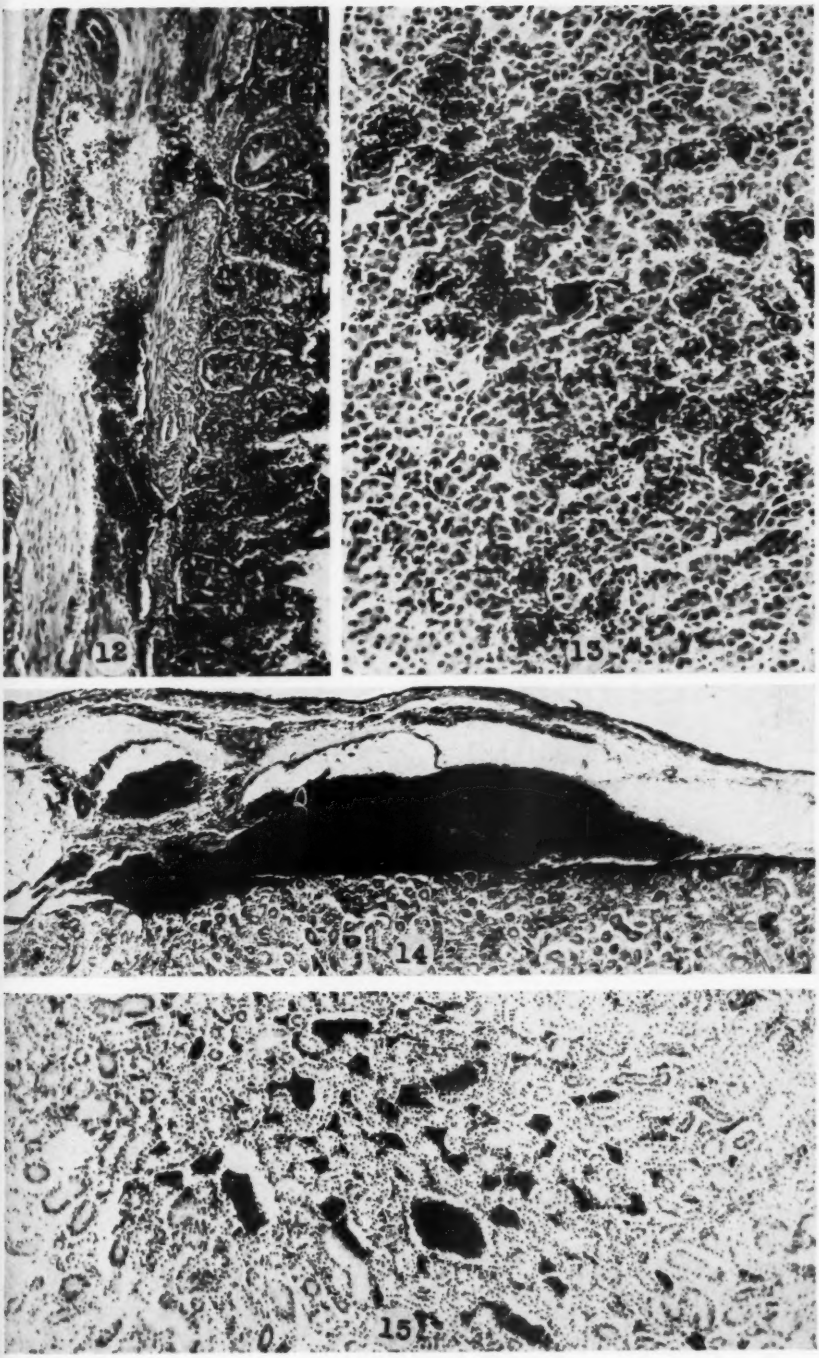
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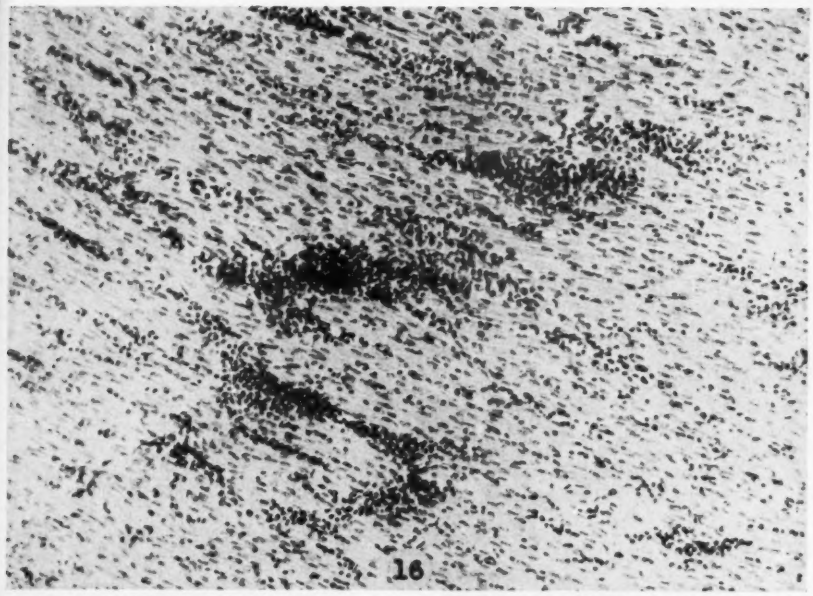
PLATE 217

FIG. 16. Hemorrhages in the myocardium of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 200.

FIG. 17. Small and large hemorrhages variously involving the pulmonary tissue of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 52.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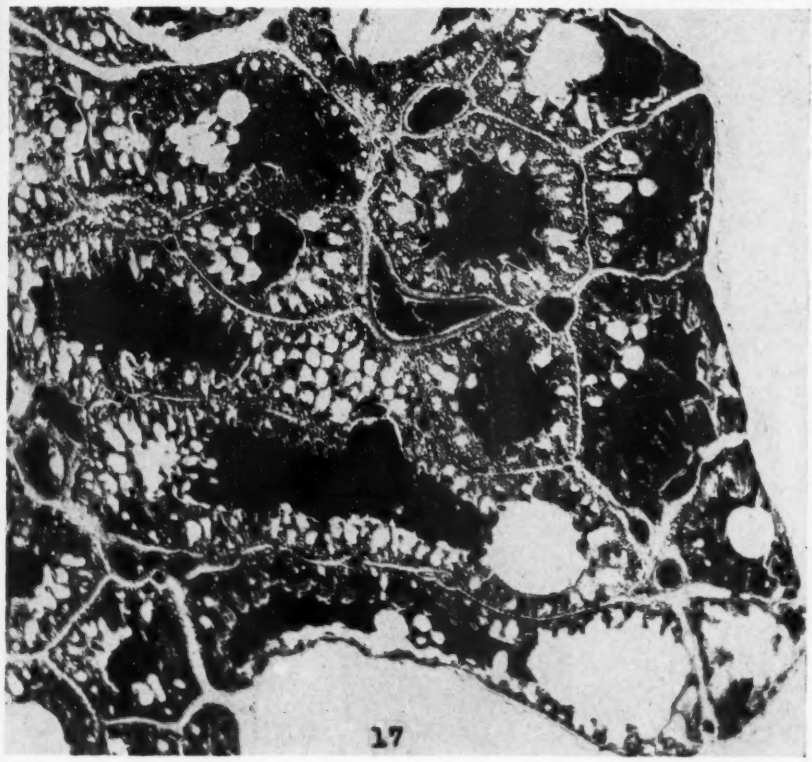
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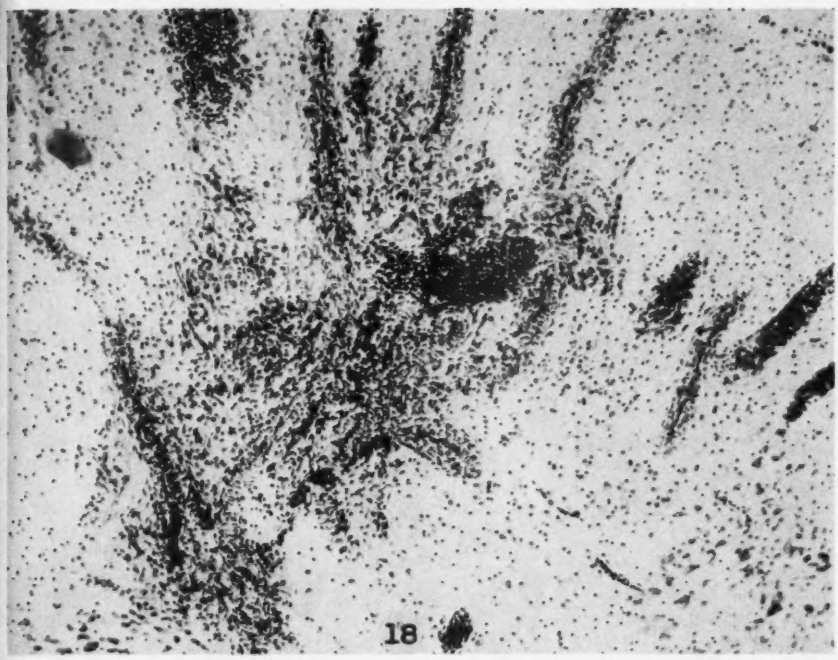
PLATE 218

FIG. 18. Central hemorrhage and congested radiating blood vessels outlined by diapedesis in the brain stem of a chick in the acute stage of vitamin K deficiency. Nissl's stain. × 100.

FIG. 19. Diffuse hemorrhages leading to severe disintegration and disorganization of liver structure in a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 140.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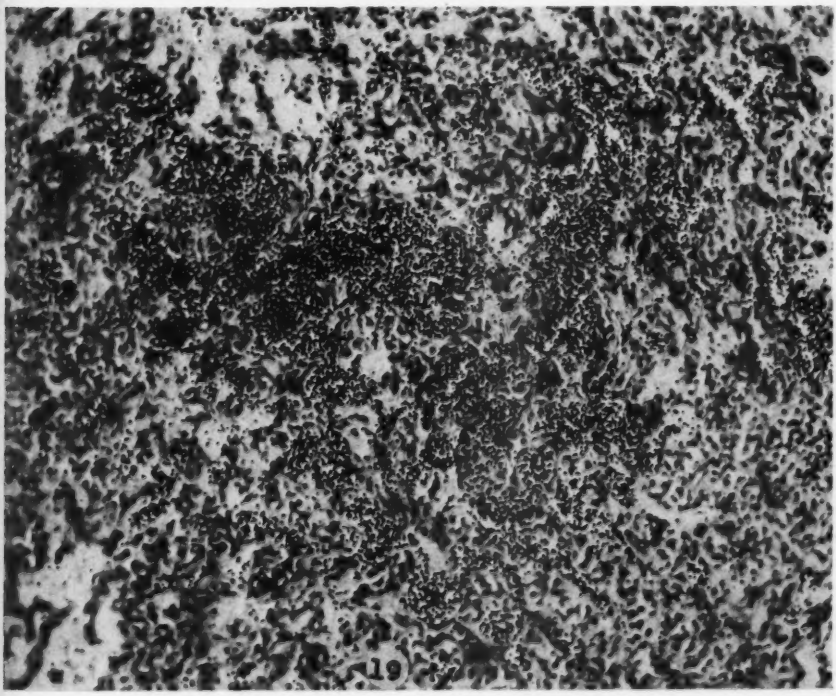
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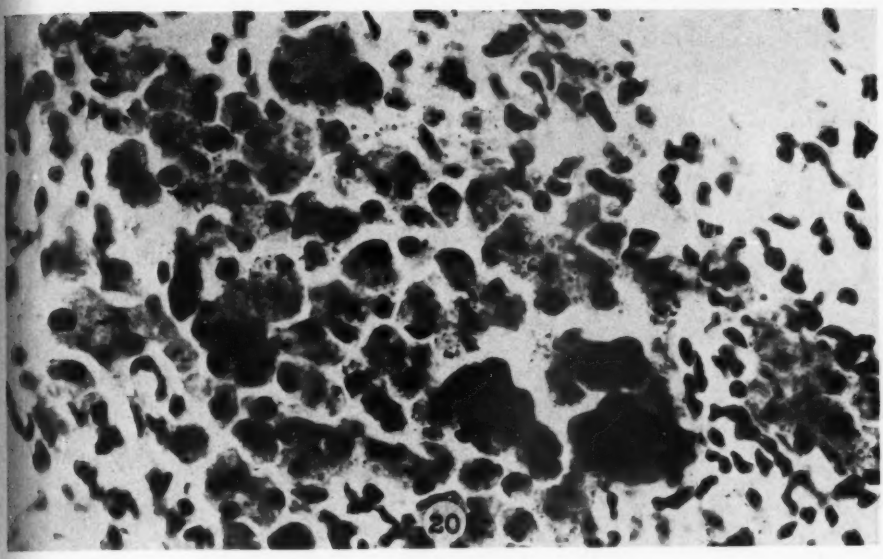
PLATE 219

FIG. 20. Large and small mononuclear phagocytic elements containing hemal granular material in the lung of a chick which died in the subacute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 560.

FIG. 21. Diffuse and marked vascular dilatation and hyperemia in the kidney of a chick sacrificed in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 34.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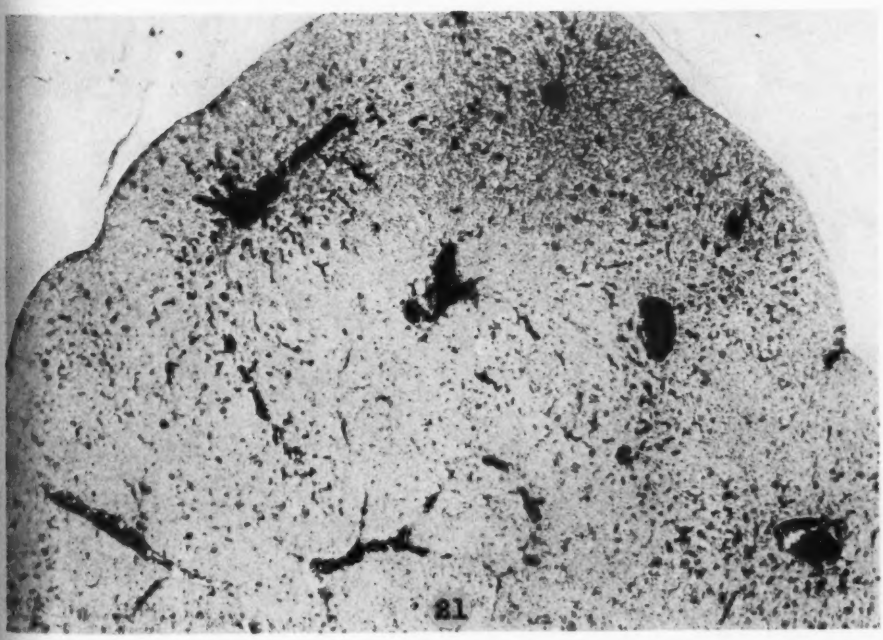
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PLATE 220

FIG. 22. Ampullar, globular, and irregular dilatation of blood vessels and capillaries in the cerebellar white matter of a chick in the acute stage of vitamin K deficiency. Nissl's stain. × 52.

FIG. 23. Small, circumscribed hemorrhages in the pons of a chick in the acute stage of vitamin K deficiency. Pickworth's method.[57b] × 52.

FIG. 24. Capillary and precapillary hemorrhages in the medulla of a chick, with tendency to diffusion, in the acute stage of vitamin K deficiency. Eros' method.[57c] × 52.

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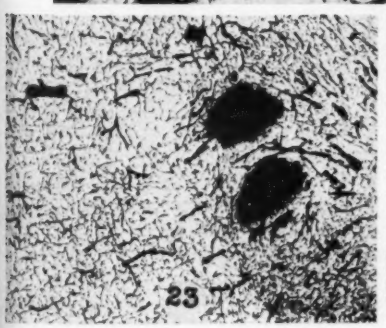
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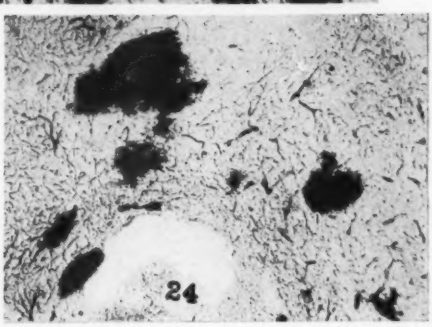
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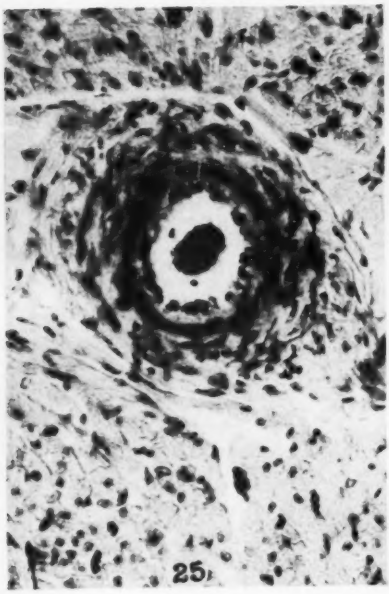
PLATE 221

FIG. 25. Edema and thickening of the walls of a myocardial artery of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain. × 200.

FIG. 26. Rupture of the walls of a cerebral artery in a chick in the acute stage of vitamin K deficiency. Weigert's elastic tissue stain. × 200.

FIG. 27. Perivascular edema and dilatation of a cerebral vein with diapedesis of blood elements in a chick in the acute stage of vitamin K deficiency. × 200.

FIG. 28. Central conglomeration of erythrocytes with peripheral displacement of leukocytes and tendency to diapedesis in a cerebral blood vessel of a chick in the acute stage of vitamin K deficiency. Nissl's stain. × 200.



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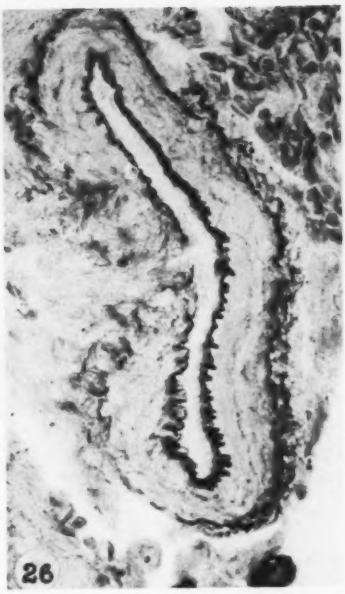

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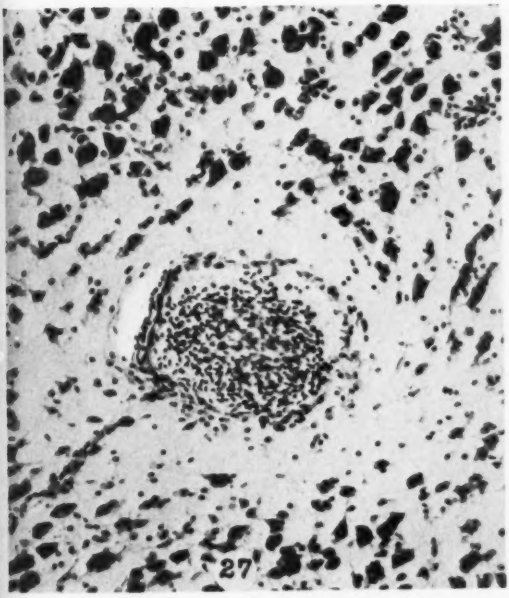

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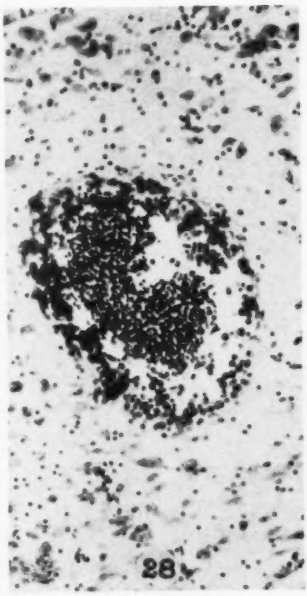

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PLATE 222

FIG. 29. Hemorrhages in the nails of the anterior extremities of a young rat in the acute stage of vitamin K deficiency.

FIG. 30. Hemorrhages in the nails of the posterior extremities of a young rat in the acute stage of vitamin K deficiency.

FIG. 31. Massive hemorrhages of the tail, toes, and nails of a young rat in the acute stage of vitamin K deficiency.

FIG. 32. Hemorrhages in the knee joints, toes, nails, and tail of a young rat in the acute stage of vitamin K deficiency.

FIG. 33. Massive hemorrhages in the subcutaneous and muscular tissues of the thorax of a young rat sacrificed in the acute stage of vitamin K deficiency.

FIG. 34. Hemorrhages in the subarachnoid space, toe, and tail of a young rat which died in the acute stage of vitamin K deficiency.

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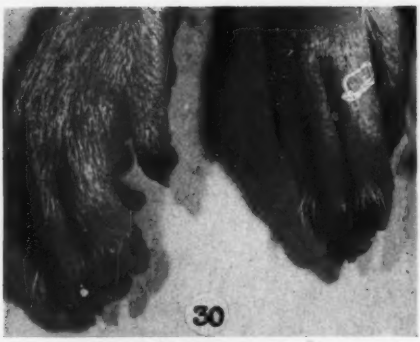
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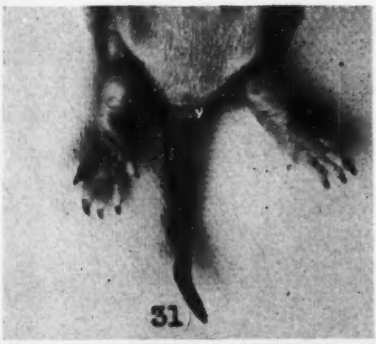
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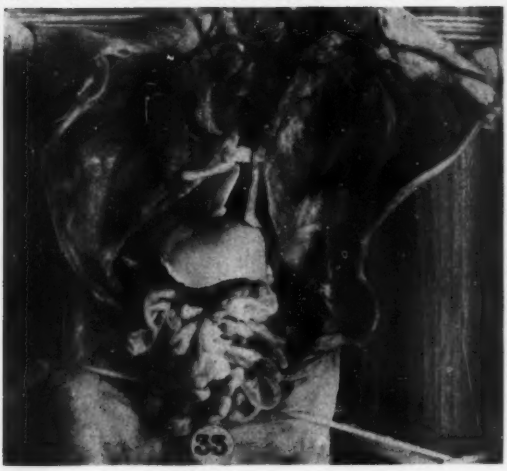
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PLATE 223

FIG. 35. Massive hemorrhages in the retro-ocular region and region of the frontal sinus of a young rat which died in the acute stage of vitamin K deficiency.

FIG. 36. Pelvic hemorrhage in a young rat sacrificed in the acute stage of vitamin K deficiency.

FIG. 37. Intraventricular and parenchymatous cerebral hemorrhages in a rat in the acute stage of vitamin K deficiency. A few hours before death the animal developed generalized tonic-clonic convulsions.

FIG. 38. Ruptured large hematoma in the subcutaneous tissue of the ventral part of the left wing of a chick in the subchronic stage of vitamin K deficiency.

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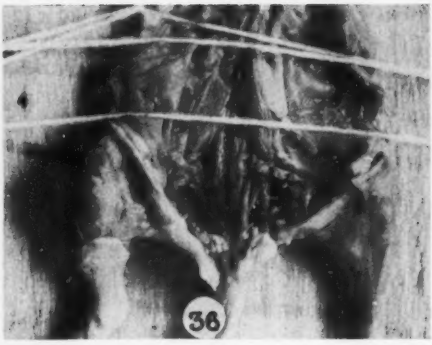
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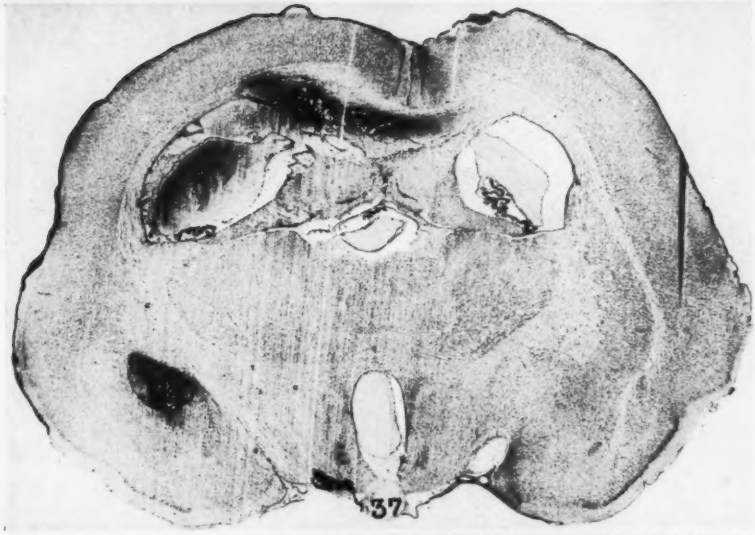
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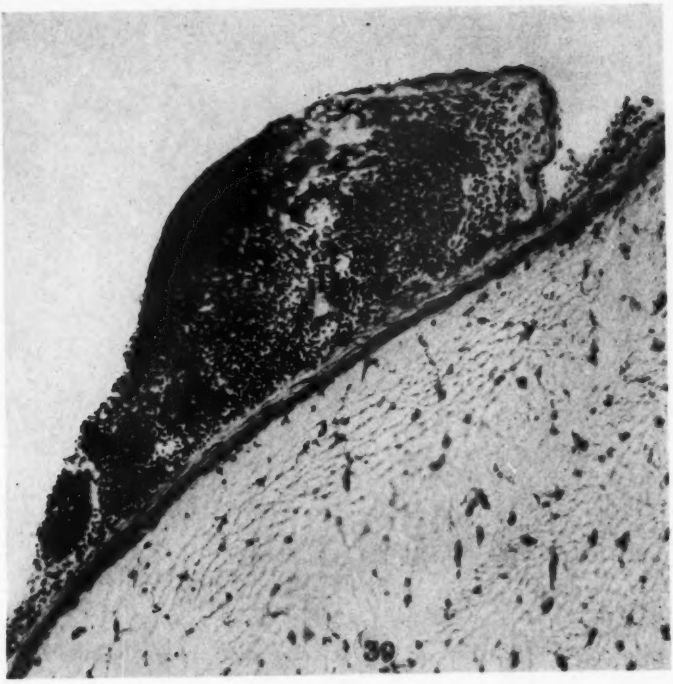
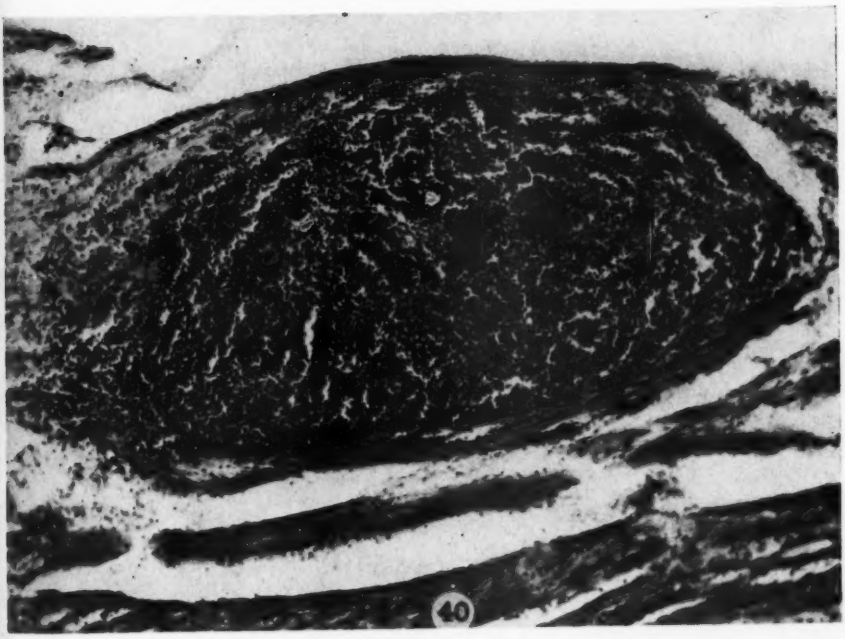
PLATE 224

FIG. 39. Intradural hematoma in the cervical region of the spinal cord of a chick sacrificed in the subchronic stage of vitamin K deficiency. Nissl's stain. × 280.

FIG. 40. Large, partially organized hematoma in the myocardium of a chick sacrificed in the subchronic stage of vitamin K deficiency. Hematoxylin and eosin stain. × 140.





Ferraro and Roizin Diathesis by Deficiency in Vitamin K

PLATE 225

FIG. 41. Fatal, massive hemorrhage in the abdominal cavity of a chick in the subchronic stage of vitamin K deficiency.

FIG. 42. Small, organized hematoma in the inner muscular layer of the gizzard of a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain. × 100.

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PLATE 226

FIG. 43. Diffuse, long-standing hemorrhage infiltrating and disintegrating the lung tissue of a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain. × 52.

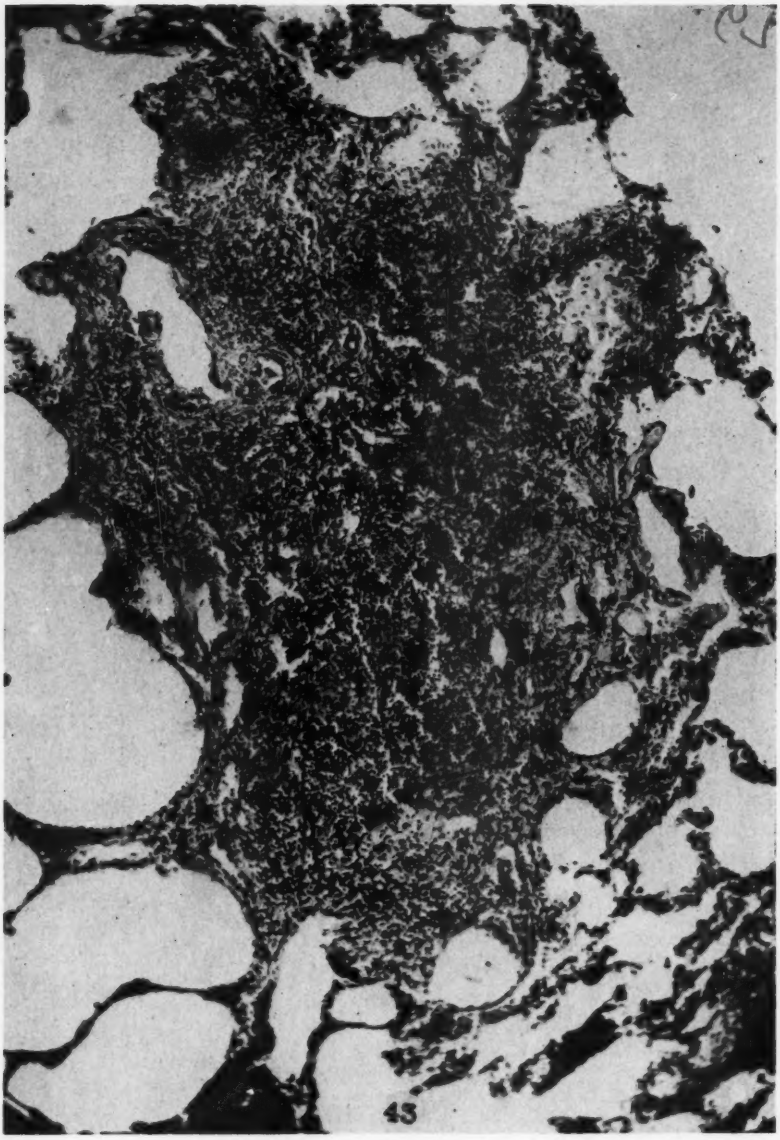
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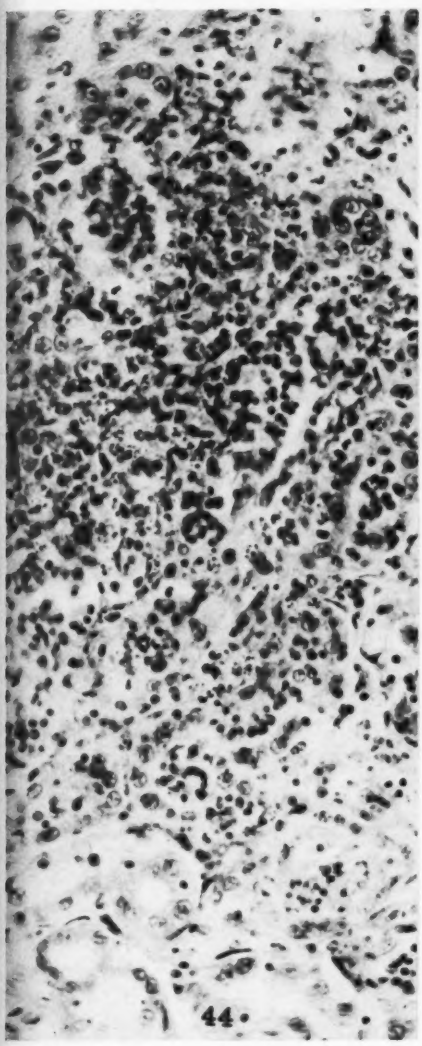
PLATE 227

FIG. 44. Amorphous granular pigment, mostly intracellular, in long-standing hemorrhage of the kidney in a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain. × 720.

FIG. 45. Compound granular corpuscles containing siderophile pigments. Chick brain in the chronic stage of vitamin K deficiency. Nissl's stain. × 720.



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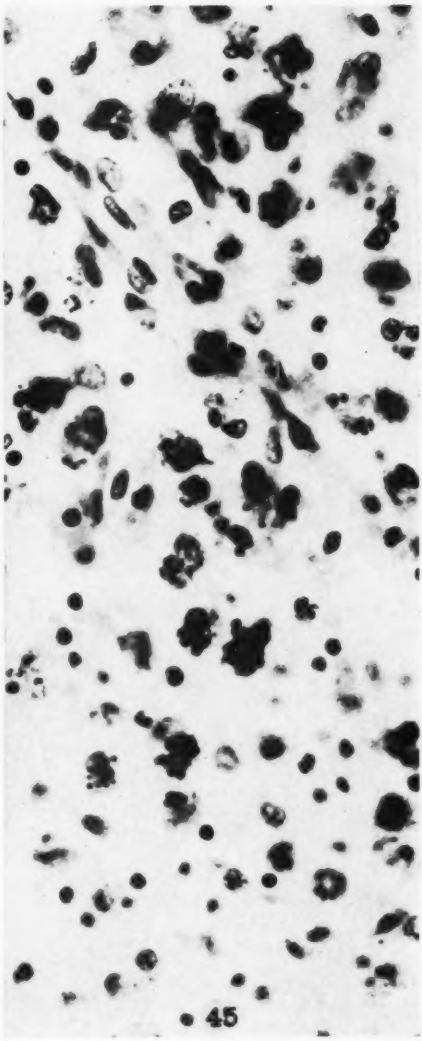

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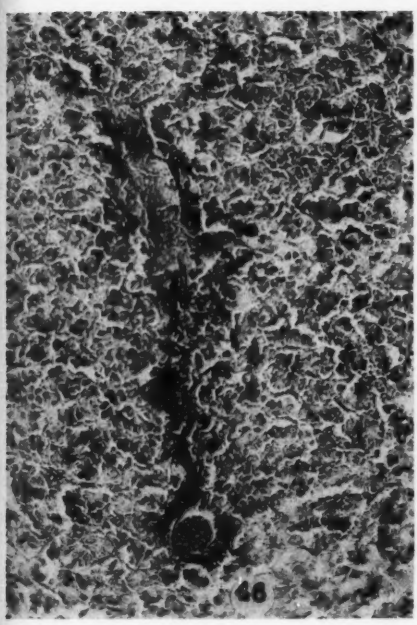
PLATE 228

FIG. 46. Fatty products of degeneration, mostly in the perivascular space. Liver of a chick in the chronic stage of vitamin K deficiency. Sudan III stain. × 140.

FIG. 47. Perivascular edema and abundant fatty material from degeneration. Myocardium of a chick in the chronic stage of vitamin K deficiency. Sudan III stain. × 100.

FIG. 48. (a) Demyelination in a cerebellar folium of a chick in the chronic stage of vitamin K deficiency. Roizin's[57a] combined method for myelin sheath and lipid products of degeneration. × 100.

(b) Perivascular cerebral demyelination in a chick in the chronic stage of vitamin K deficiency. Roizin's[57a] combined method for myelin sheath and lipid products of degeneration. × 80.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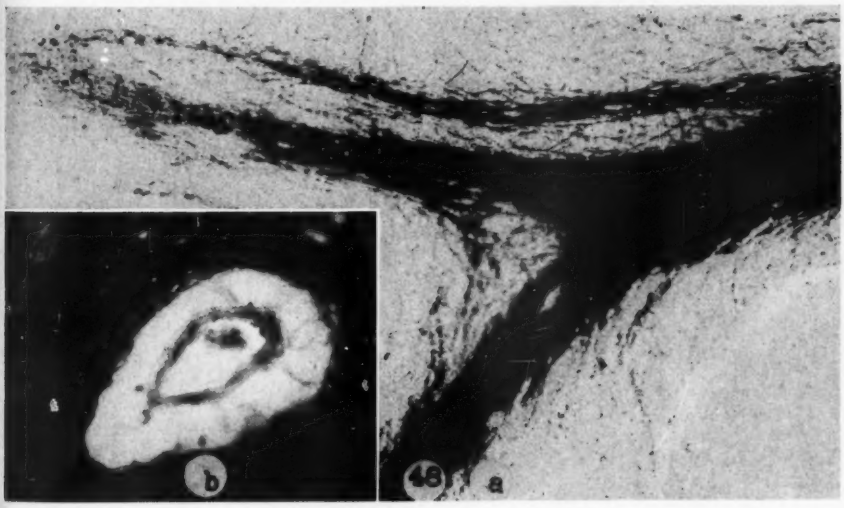
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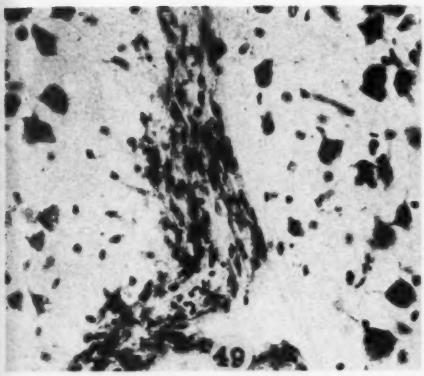
PLATE 229

FIG. 49. Stratification of endothelial vascular cells. Cerebral blood vessel of a chick in the chronic stage of vitamin K deficiency. Nissl's stain. × 190.

FIG. 50. Hyperplasia and hypertrophy of the walls of a cerebral blood vessel of a chick in the chronic stage of vitamin K deficiency. Nissl's stain. × 190.

FIG. 51. Disintegration and almost complete disorganization of liver structure, and fibrosis. Chick in the chronic stage of vitamin K deficiency. Masson's trichrome stain. × 360.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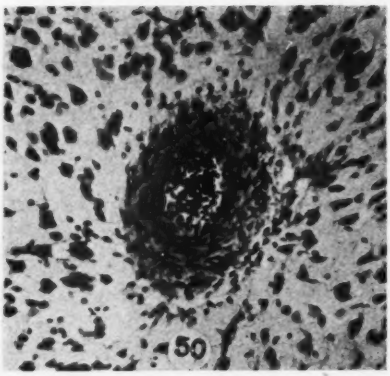
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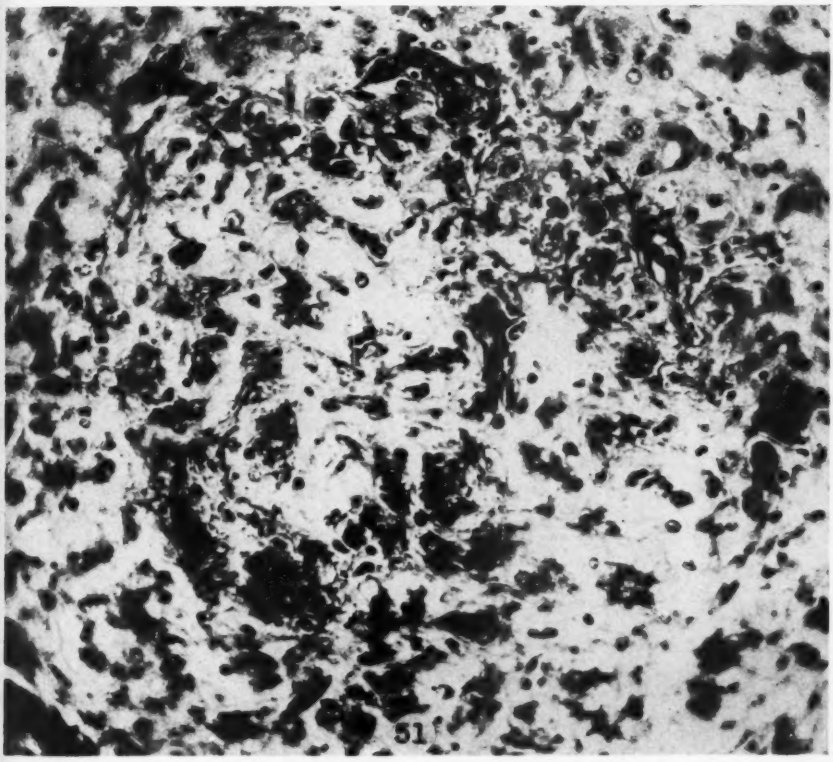
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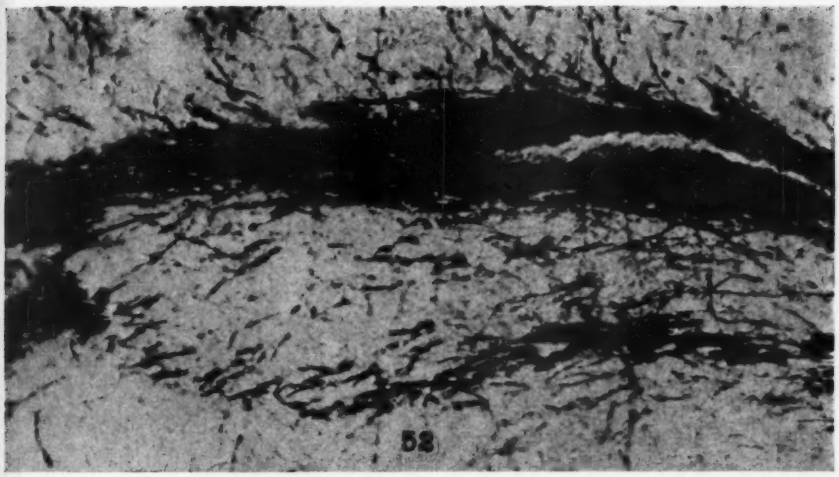
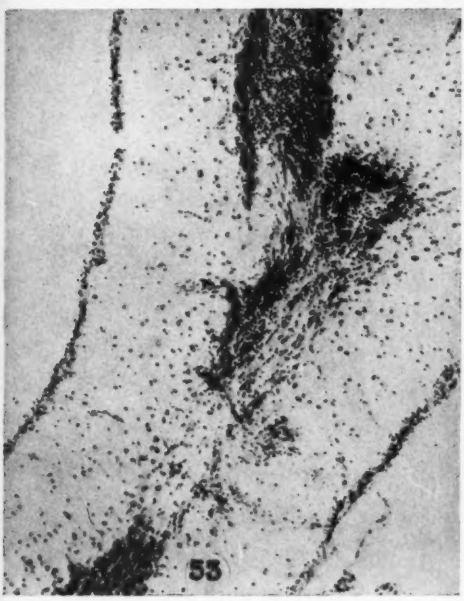
PLATE 230

FIG. 52. Demyelination and débris of disintegrated myelin sheaths in long-standing cerebellar hemorrhagic area of a chick in the chronic stage of vitamin K deficiency. Roizin's [57a] combined method for myelin sheath and lipid products of degeneration. × 190.

FIG. 53. Areas of cellular destruction in the Purkinje and granular layers of the cerebellum in a chick in the chronic stage of vitamin K deficiency. Nissl's stain. × 80.

FIG. 54. Area of destruction with remains of hemorrhage, involving mostly the granular layer of the cerebellum. Chick in the chronic stage of vitamin K deficiency. × 110.





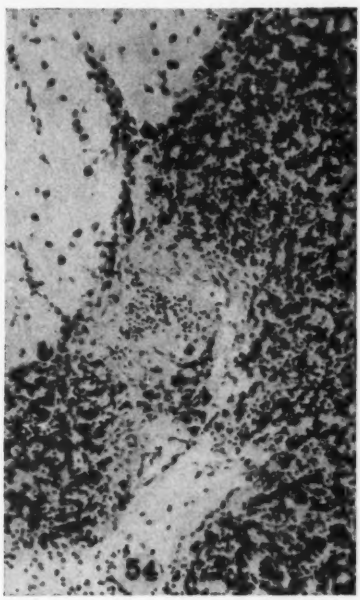
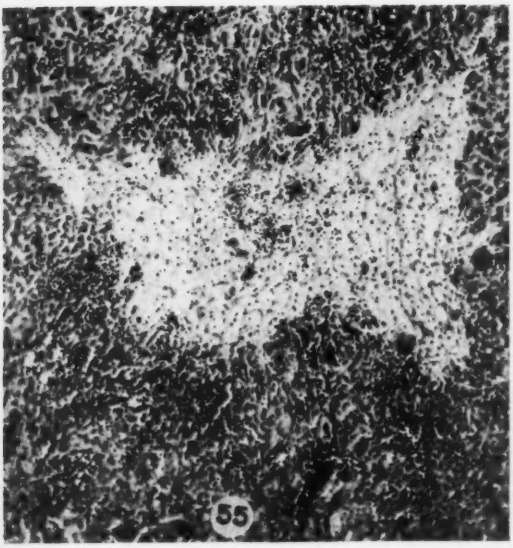


PLATE 231

FIG. 55. Area of reparative fibrosis in the liver of a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain. × 140.

FIG. 56. Small area of fibrosis with congested blood vessels in the liver of a chick sacrificed during the chronic stage of vitamin K deficiency. Masson's trichrome method. × 100.

FIG. 57. High-power magnification of Figure 56, revealing details of the fibrosis. Hematoxylin and eosin stain. × 360.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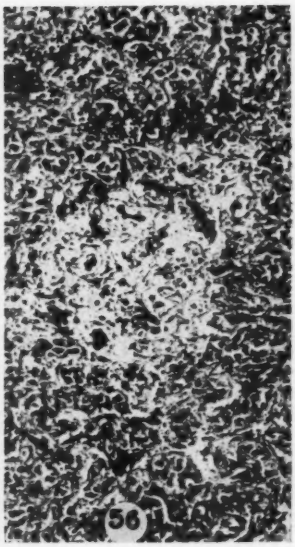
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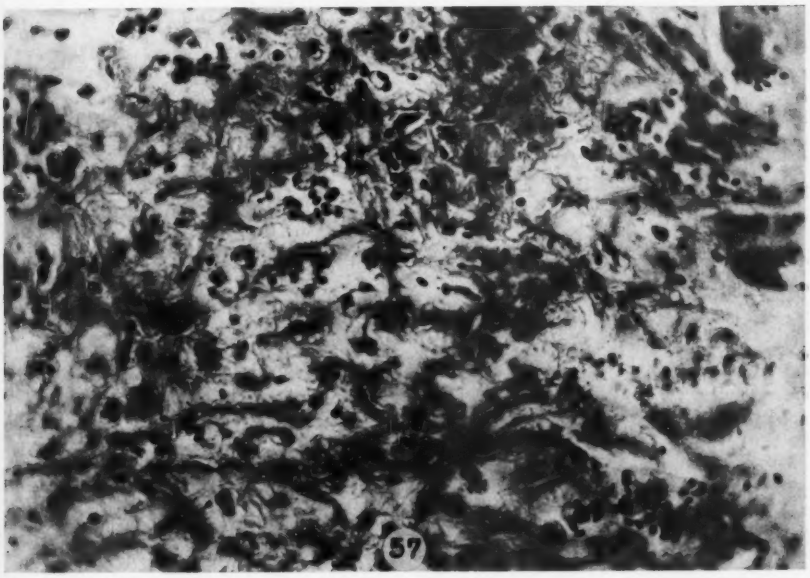
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PLATE 232

FIG. 58. Marked hypertrophy and hyperplasia of microglial cells in the central nervous tissue of a chick sacrificed during the chronic stage of vitamin K deficiency. Hortega's silver carbonate method. × 360.

FIG. 59. Hypertrophy and hyperplasia of astrocytes in an area of gliosis in a chick sacrificed in the chronic stage of vitamin K deficiency. Cajal's gold sublimate method. × 220.

FIG. 60. Organized subcutaneous hematoma of 14 months' duration in a rat sacrificed in the chronic stage of vitamin K deficiency.

FIG. 61. Small, organized hematoma in the retrosellar region of a rat (second generation), 340 days old, resulting in clinical symptoms of Fröhlich's syndrome.

FIG. 62. Marked depression at the base of the brain produced by the hematoma shown in Figure 61.

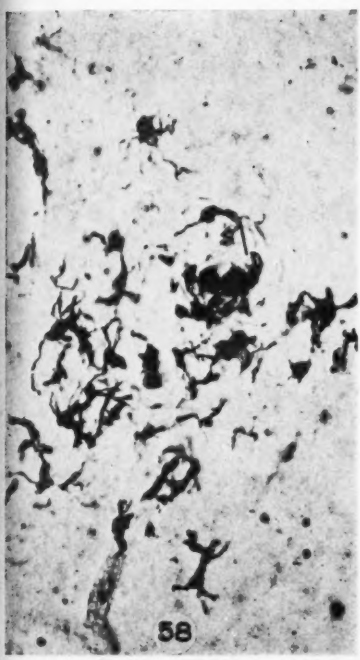
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PLATE 233

FIG. 63. (a) Marked proliferation of cerebral capillaries in a chick which died in the subchronic stage of vitamin K deficiency. Nissl's stain. × 152.

(b) Area of encephalomalacia in a chick sacrificed in the subchronic stage of vitamin K deficiency. Nissl's stain. × 360.

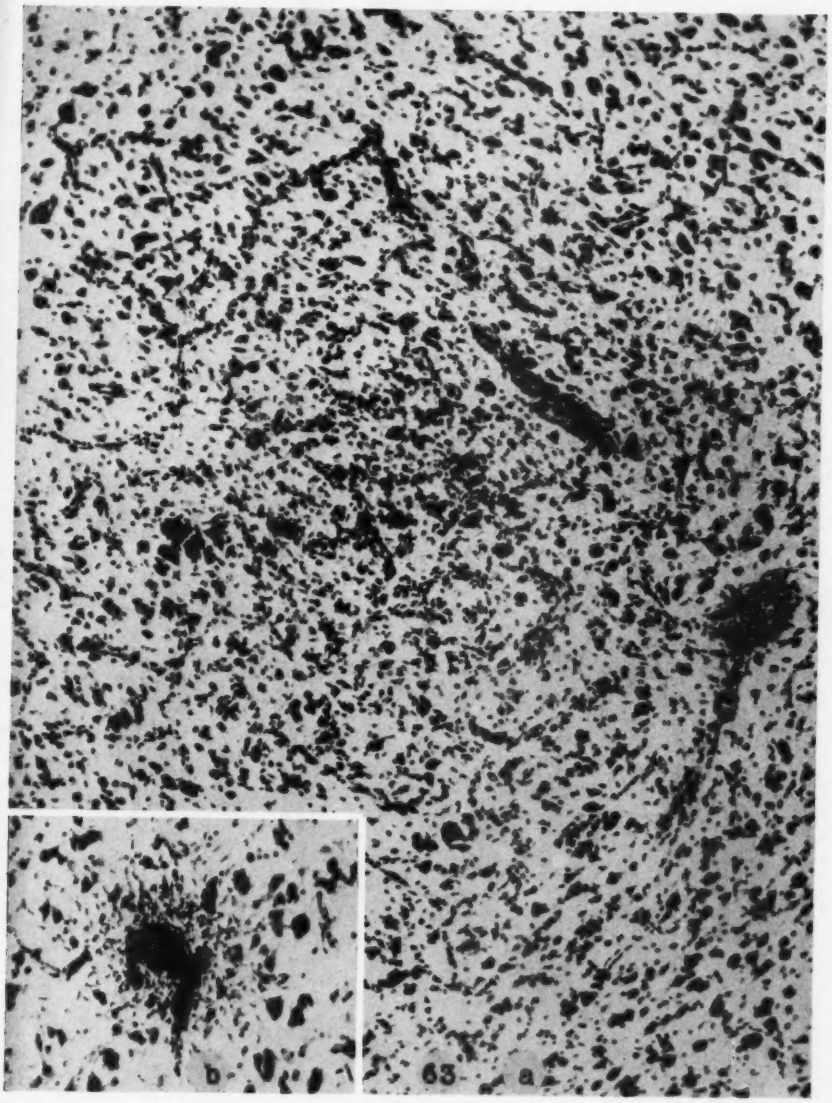
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PLATE 234

FIG. 64. Deformity of the left knee joint and retraction of the leg in flexion in the subchronic stage of vitamin K deficiency.

FIG. 65. Marked swelling of the left knee joint in the subchronic stage of vitamin K deficiency.

FIG. 66. Involvement of both knee joints resulting in inability to walk and to stand in an erect position in the chronic stage of vitamin K deficiency.

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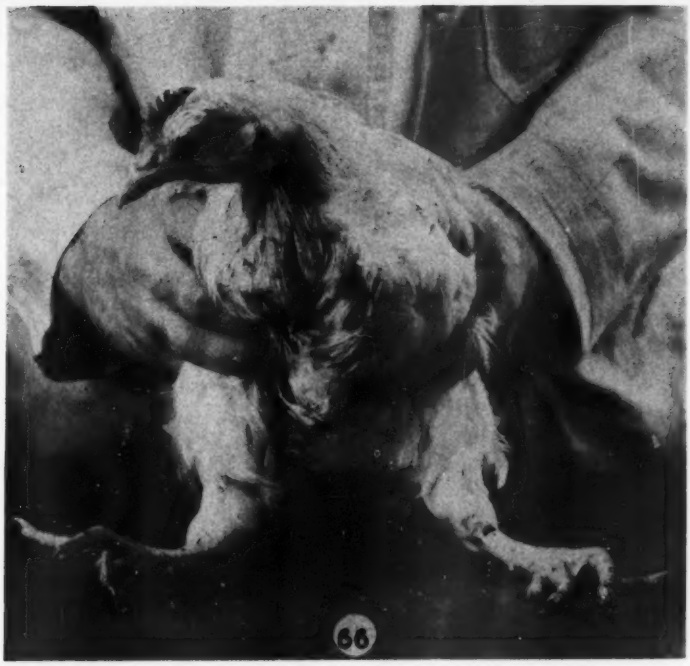
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PLATE 235

FIG. 67. (a) Lateral view of an anatomic preparation illustrating the slipping of the tendon from its normal position in a chick in the subchronic stage of vitamin K deficiency.

(b) Lateral view of an anatomic preparation illustrating the tendon of a control chick in its normal position.

FIG. 68. Posterior view of Figure 67a.

FIG. 69. Posterior view of Figure 67b.

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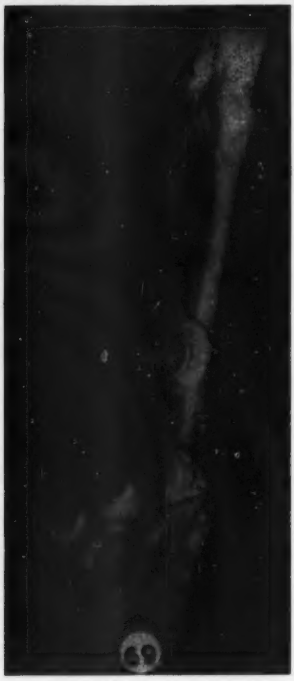
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PLATE 236

FIG. 70. Various aspects of bone deformities and dystrophies in chicks in the subchronic and chronic stages of vitamin K deficiency.

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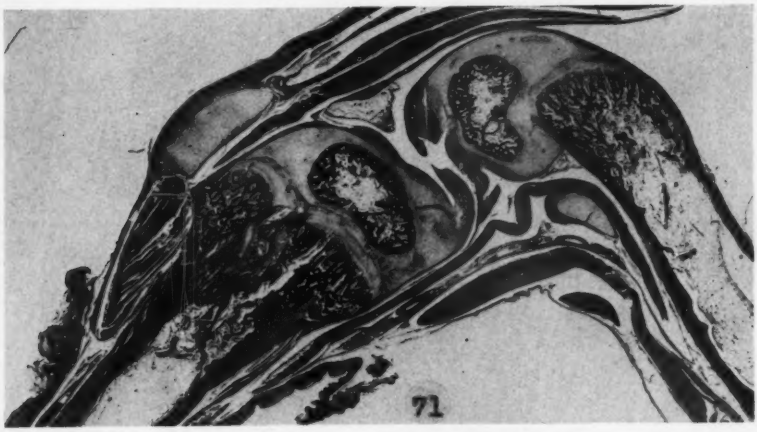
PLATE 237

FIG. 71. Deformity and rupture of periarticular and intra-articular tissues of the knee joint of a chick in the chronic stage of vitamin K deficiency. × 7.

FIG. 72. Deformity and dystrophy of epiphyseal cartilage of the knee joint of a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain. × 7.

FIG. 73. Hemorrhages in a knee joint (left side of figure) for comparison with a normal joint (right side of figure).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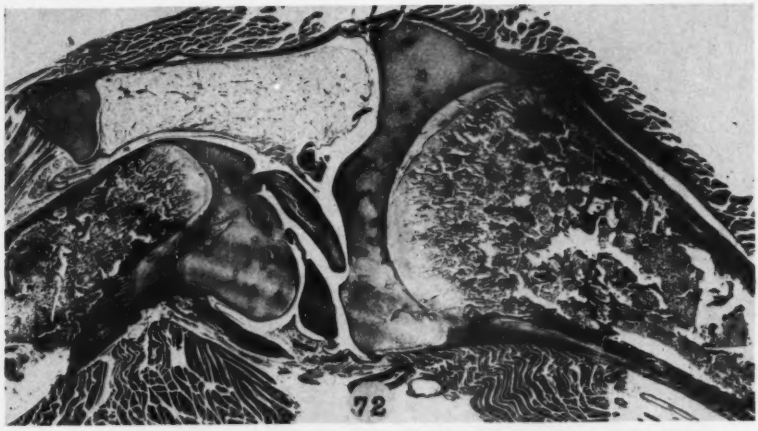
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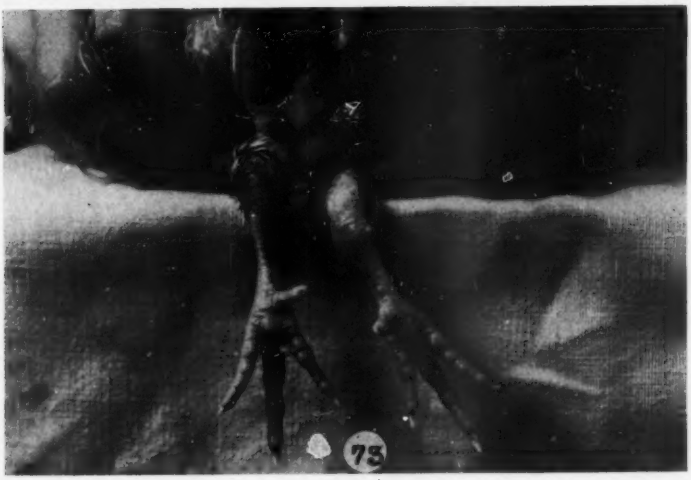
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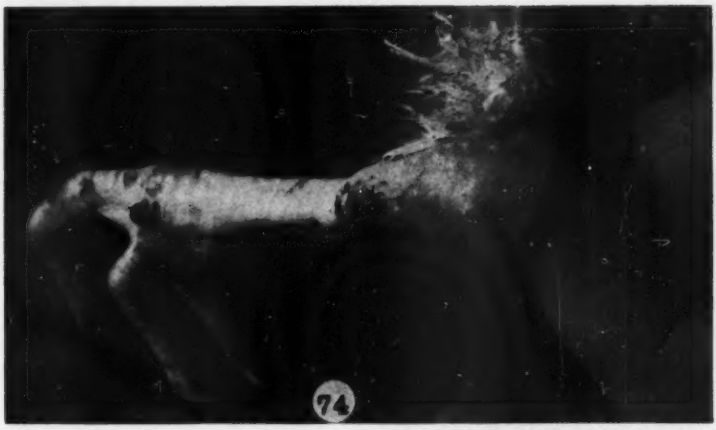
PLATE 238

FIG. 74. External swelling and distention resulting from hemorrhage in the knee joint of a chick in the subchronic stage of vitamin K deficiency.

FIG. 75. Anatomic preparation of the knee joint shown in Figure 74, revealing blood in the joint (a). The right side of the figure illustrates a normal control joint.

FIG. 76. Large hemorrhage infiltrating the upper part of the diaphysis of a tibia resulting in the fracture of the bone plate up to the periosteum in a chick in the subchronic stage of vitamin K deficiency.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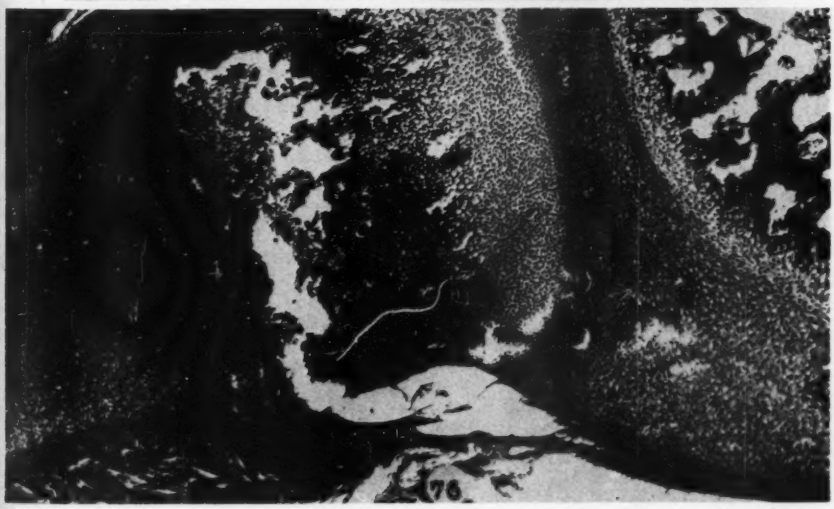
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STUDIES ON THE COAGULATION DEFECT IN A CASE OF THROMBOCYTOPENIC PURPURA COMPLICATED BY THROMBOSIS *

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Recent investigations have demonstrated that thrombosis can be prevented by the administration of anticoagulant drugs.[1] It is therefore of interest to observe a case in which thrombosis occurred in the course of a hemorrhagic syndrome featured by marked prolongation of the coagulation time of the blood. We wish to report such a case, with experimental observations on the nature of the coagulation defect, and to give a brief review of the literature dealing with the coagulation time in thrombocytopenic purpura and the neurologic complications of that condition.

The Coagulation Time in Thrombocytopenic Purpura

Although the blood platelets yield the principal source of thromboplastin for the coagulation of shed blood, there is no significant correlation between the coagulation time and the platelet count. In a group of 139 paired determinations of these factors on the venous blood in experimental thrombocytopenic purpura in dogs, Tocantins[2] found an insignificant correlation of minus 0.234 ± 0.069. In our group of 743 observations of 402 patients suffering from various diseases, the coefficient of association (Yule's Q) between the same two factors was 0.20 ± 0.16, showing no association in the group tested.[3]

In the great majority of cases reported in the literature, the coagulation time in thrombocytopenic purpura appears to have been within the normal range for the methods employed (usually under 10 minutes). However, Evans[4] found a slight prolongation of the coagulation time which returned to normal coincident with elevation of the platelet count in 8 patients who were splenectomized. Other cases in which the coagulation time was slightly or moderately prolonged (up to 25 minutes) have been observed.[4,6,7,8] Tidy[5] stated that "At present the fact is established that coagulation time is slightly, but not greatly, increased in haemorrhagic purpura. The reason for this is still uncertain."

In thrombocytopenic purpura a slight or moderate prolongation of the coagulation time is not unusual, but a *marked* prolongation has seldom been observed. Marzollo's[9] case was that of a 3-year-old girl. The platelets numbered between 10,000 and 80,000 per cmm., and the

* Received for publication, December 15, 1945.

coagulation time, tested by various methods, was 23 to 160 minutes. As clinical improvement took place, the platelet count rose to 120,000 per cmm., and the coagulation time returned to normal.

The case reported by Aubertin and Lafon [10] was that of a man, 19 years of age. Examination 1 month before death showed a markedly prolonged bleeding time and a nonretractile blood clot. The platelets numbered 136,000 per cmm. and the coagulation time was 10 minutes. Five days before death the platelets numbered 182,000 per cmm. and the coagulation time was greater than 2 hours. On the day before death the coagulation time was markedly prolonged; after standing overnight at room temperature, coagulation had taken place only in that part of the specimen occupied by the sedimented erythrocytes; the supernatant plasma remained completely liquid.

Neurologic Complications of Thrombocytopenic Purpura

Intracerebral hemorrhage has been the most frequently reported neurologic complication of thrombocytopenic purpura.[11-21] In some patients the hemorrhages have been large and solitary; in some, small and multiple; and in others they have ruptured into the subdural space or into the ventricles. In addition there have been many reports of primary subdural or subarachnoid hemorrhage.[10,18-24] Unlike hemophilia, a number of cases of cerebellar hemorrhage have been encountered in thrombocytopenic purpura.[18,20] Less commonly, hemorrhages have occurred in the midbrain,[18,25] the pituitary gland,[26] the optic tracts,[25,27] the spinal cord,[28,29] and the spinal meninges.[18] No reports of peripheral nerve involvement in thrombocytopenic purpura have come to our attention. This is in contrast to hemophilia, in which such lesions are rather frequently encountered.[30] We have found no reports indicating a disturbance in the cranial venous sinuses in thrombocytopenic purpura except in the case reported by Welt and Kasnetz [33] in which thrombocytopenic purpura occurred as a complication of acute mastoiditis with lateral sinus thrombosis, *Streptococcus viridans* bacteremia, and multiple metastatic abscesses.

Report of Case *

W. J. F. (no. U 79071) was a white male machine operator, 29 years of age, who entered the University of California Hospital on December 4, 1941.

History. The family history was irrelevant for bleeding diseases. No cause of secondary thrombocytopenic purpura was discovered in the past history. At the age of 19 years the patient had suffered from toxic hepatitis following the administration of neoarsphenamine given for an indolent ulcer of the left lower leg. The ulcer persisted, and in 1939 and 1940 additional ulcerated areas appeared on the dorsal surfaces of both feet. In recent months the lesions had healed, leaving

* A brief summary of this case has been published previously.[40]

heavily pigmented scars. He had had a nonproductive cough for many years and for the past 3 years had suffered from dyspnea on exertion, ankle edema, and nocturia. For the past 6 months he had experienced increasing "stiffness" of the leg muscles. The patient had bruised easily since childhood. During the past year bouts of epistaxis had occurred with increasing frequency and severity and numerous petechial hemorrhages had appeared in successive showers over the lower extremities. He had also noted bright red blood in the stool on numerous occasions.

Physical Examination. The patient was undernourished, fairly well built, pallid, and appeared older than his stated age of 29 years. There was a generalized purpuric eruption, most marked over the lower extremities and characterized by innumerable petechiae and many purpuric maculae as large as 1 cm. in diameter. There were large scarred areas over the lower part of the left foreleg and ankle and over the dorsa of both feet, where the skin was thin, scaly, deeply pigmented, and fixed to the underlying tissues. The lymph nodes were not enlarged. In the right optic fundus there was a large, fresh hemorrhage and several areas of absorbing exudate. The nasal passages were obstructed with crusted blood and there was continuous oozing from the right nostril. The mouth was edentulous and there were numerous small hemorrhages in the oral and pharyngeal mucous membrane. The lungs and heart were within normal limits. The pulse rate was 100 per minute and the blood pressure was 150/95 mm. Hg. The peripheral arteries were soft and pulsated normally. The spleen descended two fingersbreadth below the left costal margin, but no other abdominal organs or masses were palpable. The right testicle was in the inguinal canal. Prolapsing internal hemorrhoids were found on rectal examination. The fingers were slightly clubbed and there was slight thoracic kyphosis, but the remainder of the skeletal system was normal. Examination of the nervous system revealed no abnormalities.

Laboratory Findings. The hematologic findings are given in Table I, and normal values for the hemostatic tests employed are given in Table II. There was a marked anemia, probably due to continuous blood loss. The leukocyte count varied between 2,900 and 6,400 per cmm. The differential count was normal except for a slight increase in the percentage of polymorphonuclear neutrophils. The platelet count varied between 80,000 and 250,000 per cmm. Furthermore, it was noted that the platelets were large, stained poorly, and did not contain granules. The bleeding time was markedly prolonged, the clot retraction very poor, and the capillary fragility definitely increased. The prothrombin concentration was slightly below normal on the patient's entry to the hospital and fell to 40 per cent of normal during the ensuing 3 weeks. It rapidly returned to normal following the administration of vitamin K. The coagulation time of the venous blood, tested at room temperature, was moderately prolonged when the patient was first observed and subsequently became markedly prolonged. The coagulation time of the venous blood at 37° C. was tested only during the last week of life when it was found to be moderately prolonged.

It should be pointed out that, while there is only a slight difference between the normal values for the coagulation time at room temperature and at 37° C., there is a marked difference between their pathologic ranges. In parallel tests done on the same blood specimens it has been found that coagulation time of 5 to 10 hours at room temperature will be reduced to 1 to 2 hours at 37° C.[44] The experimental observations relating to the coagulation defect were made on January 5 and 7, 1942, and are discussed below.

The urine was grossly bloody, specific gravity was 1.023, albumin was positive (3 plus). The stool gave a positive test for occult blood (4 plus). The plasma

TABLE I
Results of Hematologic Tests

Date	Hemoglobin in gm. per 100 cc.	Erythrocytes in millions per cmm.	Leukocytes per cmm.	Differential white blood cell count in per cent					Platelets per cmm.	Bleeding time in minutes
				Polymorphonuclear cells		Polymorphonuclear eosinophils	Lymphocytes	Monocytes		
				Fil.	Non-Fil.					
12-4-41	7.7	2.68	5300	60	4		20	16	80,000	
12-5-41									70,000	30
12-10-41									90,000	
12-14-41									100,000	
12-16-41	6.1	1.88	4300	74	8	1	10	7	180,000	
12-20-41			4600						170,000	
12-21-41									190,000	
12-23-41										
12-24-41	6.9	2.53	4300						160,000	10½
12-26-41	6.9	2.50	3900						170,000	
12-27-41	7.4	2.58	3200						120,000	
12-29-41	7.7	2.45	4400						120,000	
12-30-41	7.7	2.60	3100						230,000	30
12-31-41	8.4	2.74	3400						200,000	
1-2-42	8.4	3.01	3900						250,000	30
1-3-42	7.6	2.78	2900						200,000	
1-5-42	7.7	3.01	3700						160,000	
1-6-42	7.7	3.22	4500						180,000	30
1-7-42									100,000	
1-8-42	7.2	2.70	4900	46	35		11	8	140,000	

TABLE I—*continued*

Date	Coagulation time of venous blood at room temperature in minutes	Coagulation time of venous blood at 37°C. in minutes	Fluid volume of clot in per cent	Prothrombin concentration in per cent of normal	Capillary fragility in numbers of petechiae					Citrated whole blood transfusions in cc.
					Arm		Thigh			
					mm. Hg suction		mm. Hg suction			
					150	200	100	150	200	
12-4-41										
12-5-41	24		50	75	15	Shower*	Shower	Shower	Shower	300
12-10-41										500
12-14-41										500
12-16-41										
12-20-41										
12-21-41										500
12-23-41										500
12-24-41	42		44	40	2	2	1	Shower	Shower	
12-26-41										
12-27-41										500
12-29-41										
12-30-41	105		43	45†	1	4	6	Shower	Shower	
12-31-41										
1-2-42	175		43	70	0	0	Shower	Shower	Shower	
1-3-42										500
1-5-42		23½								
1-6-42	130		44	80	9	10	Shower	Shower	Shower	
1-7-42		25½								500
1-8-42	52	24½								500

* "Shower" indicates that the petechiae were too numerous to count accurately; usually from 50 to several hundred were present.

† Two mg. Synkamin (4 amino-2 methyl-1 naphthol) Parke, Davis & Co., administered intravenously on 12-30-41 and daily thereafter.

fibrinogen was 1.17 gm. per 100 cc. The serum calcium was 9.6 mg., serum phosphorus, 5.43 mg., and nonprotein-nitrogen of the blood, 32.9 mg. per 100 cc. The Wassermann and Kahn reactions of the blood were negative. The icterus index was 9 units and the intravenous hippuric acid test for liver function gave a normal value (1.09 gm.). The intravenous phenolsulfonphthalein test of kidney function showed 70 per cent excretion of the dye in 2 hours. The tuberculin (1:10,000) skin test was negative. The patient belonged to blood group A.

Course of Illness. The patient received 4,300 cc. of citrated blood by transfusion (Table I). Numerous thrombi developed in the vessels used for venipuncture. Between December 9 and December 29, 1941, the patient received roentgen irradiation of the spleen (a total of 1200 r. to each of two areas). Epistaxis continued to recur despite repeated nasal packing and cauterization of bleeding points in Kiesselbach's area. The urine and stool were grossly bloody throughout most of his hospital stay. On December 8th the right malar region became edematous and markedly tender. Roentgenograms showed complete opacity of the right antrum and ethmoid cells without evidence of bone destruction. The condition persisted for about a week and was interpreted as due to hemorrhage into the antrum. On December 19th the patient complained of sore throat and difficulty in swallowing, and he was found to have a large submucous hemorrhage in the posterior wall of the pharynx, extending down into the tissues of the neck. While this hemorrhage was resorbing, he began to have a daily temperature elevation, reaching a maximum of 41°C. (rectal) on December 22nd. Although no abnormal physical signs were detected in the chest, roentgenograms showed patchy areas of increased density throughout most of the right lung field. During the following 8 days a total of 42 gm. of sulfadiazine were administered orally and the temperature gradually returned to normal. On January 6, 1942, the patient complained of pain

TABLE II

*Normal Values for Hemostatic Tests**

Test	Mean	Standard deviation	Normal range
Bleeding time (Ivy), in minutes	3.2	1.6	0 to 6.4
Coagulation time of venous blood at room temperature in minutes	8.9	2.7	3.5 to 14.3
Coagulation time of venous blood at 37°C. in minutes	7.5	1.4	4.7 to 10.3
Clot retraction. Fluid volume per cent of clot†	7.9	6.0	4.1 to 19.9
Platelet count per cmm.	409,000	68,000	273,000 to 545,000
Prothrombin concentration (Quick) in per cent of normal			75 to 100
Capillary fragility (Dalldorf). Number of petechiae appearing on arm with 200 mm. Hg vacuum pressure			0 to 10

* The coagulation time at 37°C. was determined on a group of 40 normal subjects. All other tests were performed on a group of 64 normal subjects. (This is the same group used by us in previous analyses [49,50] except that in a more critical clinical analysis 36 subjects, formerly considered to be normal, were excluded because their normality was questioned. This resulted in no statistically significant differences in the values for the mean, standard deviation, or normal range.) The standard statistical method was used in setting up the limits of normality for the bleeding time, coagulation time, fluid volume of the clot, and platelet count. The limits of significance of the data were set at two standard deviations from the mean, thereby including approximately 96% of the observations. The mean is taken at the point of reference. All measures which are calculated were at least three times their sampling errors. The limits of normality for the prothrombin concentration and capillary fragility were arbitrarily set by direct observation. The prothrombin concentration in 91% of the observations fell between 75% and 100%. In the capillary fragility test not more than 10 petechiae appeared in 92% of the observations. The technics for the tests here employed are described in references 34, 43, 48, and 49.

† Formerly called the "Extracorpuscular volume per cent" of the clot.

in the right upper abdominal quadrant, which by the following day had extended into the right lower chest and was referred to the right shoulder. On the evening of January 10th the patient complained of marked headache over the vertex. His neck was stiff and painful on attempted flexion, but he had no other abnormal neurologic signs. By the following morning he was in coma and had moderate bilateral symmetric proptosis, and marked boggy edema of the entire scalp, nuchal region, forehead, eyelids, and tongue. At 2:00 P.M. lumbar puncture was performed. The initial pressure was greater than 1,000 cm. of water, and after removal of 70 cc. of grossly bloody fluid the pressure was 750 cm. of water. There was no immediate change in the patient's condition and he expired 1 hour later.

AUTOPSY FINDINGS

Autopsy was performed 2 hours after death. Post-mortem lividity had appeared, but rigor mortis was absent. The entire scalp was thickened, boggy, and edematous, and the coronal portion was infiltrated with blood. There was marked edema of the eyelids, the under side of the tongue, and the posterior portion of the neck. There was moderate symmetric proptosis of the eyes, which were deviated upwards and divergent.

There was a small area of hemorrhage in the fat of the anterior mediastinum. The right pleural cavity contained 750 cc. of yellow, turbid fluid. There were numerous subpleural hemorrhages, bilaterally. The largest (8 cm. in diameter) was beneath the right posterior basal parietal pleura and the pleural membrane adjacent to it was thickened. The right lung weighed 500 gm.; the left lung, 300 gm. The major bronchi and pulmonary vessels were normal. Both lungs were congested and edematous, but there was no gross hemorrhage or consolidation.

The peritoneal cavity was normal. A portion of the omentum was adherent to the gallbladder. The liver weighed 2,200 gm. The anterior edge was rounded and the cut surface pale. The gallbladder and bile ducts were normal. The spleen weighed 340 gm.; its capsule was reddish gray and slightly wrinkled; numerous large, white lymphoid follicles were visible on the cut surface. The splenic pulp was pale red and could be scraped readily from the cut surface. The pancreas was normal. The entire gastrointestinal tract was normal except for a few tiny, mucosal hemorrhages in the stomach. The kidneys each weighed 260 gm. The corticomedullary differentiation was indistinct. Numerous small (1 to 2 mm.) hemorrhages were present throughout the parenchyma and beneath the capsule. The renal pelves and calyces, ureters, bladder, and prostate gland were normal. The undescended right testicle was half the normal size.

The heart weighed 340 gm. and was normal except for hemorrhagic infiltration of the right auricular wall and numerous atheromatous

plaques in the coronary arteries, one of which, situated 1 cm. from the mouth of the left coronary artery, partially occluded its lumen. There were a few small, atheromatous plaques in the abdominal aorta.

The pituitary body, thyroid gland, and adrenal glands were normal. There was an irregular, small (0.5 cm.) polypoid mass, containing several small mucoid cysts, projecting from the left vocal cord. The mediastinal and aortic lymph nodes were moderately enlarged, soft and congested. The marrow of the ribs and lumbar spine and sternum appeared grossly normal.

The brain weighed 1,310 gm. The cerebral convolutions were flattened and the cerebellar tonsils were prominent. There was an extensive subarachnoid hemorrhage around the base of the brain with a small amount of blood over the cortex. No other abnormalities were noted in the brain, but both cavernous sinuses were completely filled with firm, adherent, thrombotic material. The venous sinuses at the base of the skull were partly occluded by similar thrombi.

Microscopic Examination

Only those tissues which showed histopathologic changes will be described in detail. The heart, liver, gallbladder, pancreas, gastroenteric tract, and thyroid, parathyroid, prostate, and thymus glands were normal.

The pulmonary alveoli contained large numbers of pigment-filled macrophages. In some there were masses of condensed, hyalinized fibrin with early organization and many were atelectatic. A section of the right posterior basal parietal pleura showed that the pleura, subpleural connective tissue, and adjacent muscle were edematous and infiltrated with red blood cells and masses of hyaline fibrin with early organization.

The epithelium of the larynx was elevated. In the submucosa there was extensive infiltration with fresh red blood corpuscles and masses of hyaline fibrin and one zone of vascular granulation tissue containing pigment-filled macrophages. A majority of the numerous large veins contained organizing thrombi.

The malpighian bodies of the spleen were not enlarged nor well outlined. In the walls and in the lumina of the sinuses, reticulo-endothelial cells and macrophages were unusually prominent, and in the follicles these cells had replaced most of the lymphocytes. Both the sinuses and intersinusoidal spaces contained a moderate amount of blood, many polymorphonuclear leukocytes, a few neutrophilic myelocytes and plasma cells, a rare megakaryocyte, but no nucleated red blood corpuscles. No phagocytosis was demonstrable.

In the kidneys the majority of the glomeruli were normal; some were partially or completely hyalinized; some presented pericapsular fibrin deposits; and a few showed epithelial or fibrous capsular crescents. The afferent arterioles of occasional glomeruli were filled with hyaline thrombi. There were numerous, small, interstitial hemorrhages both recent and old, and many tubules contained blood or serous fluid. Collections of interstitial lymphocytes and small calcium deposits were noted.

There were a few small hemorrhages in the submucosa of the bladder which was infiltrated with many large lymphocytes and macrophages and a smaller number of neutrophilic leukocytes and plasma cells. There was considerable tubular atrophy of the right testis; however, interstitial cells were present in normal numbers. The urethral submucosa was moderately infiltrated with lymphocytes, large macrophages, and neutrophilic leukocytes.

The pituitary gland showed moderate hemorrhage into the capsule and the posterior lobe.

There was a small, recent, cortical hemorrhage in one adrenal gland.

Epidermis from the ankle was normal. The dermis was composed of dense, hyalinized, acellular fibrous tissue containing scattered lymphocytes and pigment-filled macrophages.

Mediastinal, peri-aortic, and other lymph nodes had essentially the same histologic structure. The peripheral follicles were large and fairly distinct. Except for a rim of lymphocytes, they consisted of large numbers of reticulo-endothelial cells and macrophages, a few plasma cells, and many nuclear fragments. The sinuses were prominent and dilated, and there was proliferation of the lining reticulo-endothelial cells. In the sinusoids there were numerous large macrophages, many of which contained blood pigment, and fewer lymphocytes, mast cells, plasma cells, and eosinophilic and neutrophilic leukocytes.

The relative quantity of fat cells, and of erythropoietic and myelopoietic tissue in the bone marrow was normal (Fig. 1). Approximately 20 per cent of the megakaryocytes had large, well formed, vesicular, multilobar nuclei and abundant, finely granular, eosinophilic cytoplasm without pseudopods. There was moderate shrinkage of the cytoplasm and nucleus of about 40 per cent of the megakaryocytes, and the remaining 40 per cent consisted of irregular, shrunken, basophilic nuclear masses surrounded by extremely scanty cytoplasm. An average of one megakaryocyte was present per high-power field.

No histologic alteration of the central nervous system was noted except for the presence of red blood cells and blood-pigment-containing macrophages in the subarachnoid space.

The fibrous septa, nerves, and ganglia of the cavernous sinuses were infiltrated with blood which had become condensed and amorphous. A surrounding chronic inflammatory reaction had occurred. The venous channels were narrowed and almost all of them were filled with thrombi (Figs. 2 and 3). The older thrombotic masses were composed of hyalinized fibrin and red blood corpuscles. Many of these thrombi had undergone early organization. The most recently formed thrombi consisted of platelet masses containing leukocytes and erythrocytes (Fig. 4). There was no apparent primary alteration of the endothelium.

An atherosclerotic process with calcification had greatly narrowed the lumina of both branches of the left coronary artery. There was minimal atherosclerosis of the aorta. There was marked alteration of small arteries (50 to 200 μ) in the myocardium, lungs, kidneys, gallbladder, urinary bladder, spleen, and in the capsules of the pituitary and adrenal glands. Some of these vessels showed hyperplasia of the media while others presented a fibrous intimal thickening. An occasional vessel of this size had a hyalinized wall, but none contained thrombi. The arterioles were not altered.

Pathologic Diagnoses

(1) Primary thrombocytopenic purpura with hemorrhage in the subarachnoid space, in the fibrous septa and nerves and ganglia of the cavernous sinuses, and in the mediastinum, lungs, subpleural space, right auricle, kidneys, stomach, adrenal gland, vocal cord, scalp, and skin. (2) Thrombosis of the basilar and cavernous sinuses, and of veins in the arms and laryngeal submucosa. (3) Generalized arteriosclerosis with atherosclerosis of coronary arteries and aorta, and hyperplastic arteriosclerosis involving small arteries of the myocardium, lungs, gallbladder, and the capsules of the pituitary and adrenal glands.

Discussion

The Hemostatic Defect

The clinical manifestations of hemorrhagic diathesis in this patient were those commonly found in purpura haemorrhagica and the results of the hemostatic tests were characteristic of that condition; *i.e.*, a low platelet count associated with markedly prolonged bleeding time, diminished blood clot retraction, and increased capillary fragility. In addition, the coagulation time of the blood was markedly prolonged. The most probable cause of the coagulation defect was hypothromboplastinemia. However, detailed investigations were performed in order

to exclude all other known causes of delayed coagulation of the blood.

Hypocalcemia was readily eliminated as a diagnostic possibility by the finding of a normal concentration of the serum calcium and by the inability of added calcium chloride solution to shorten the coagulation time.

A defect in coagulation due to deficiency of fibrinogen was likewise excluded by the finding of a high plasma fibrinogen concentration and by the appearance of ample quantities of formed fibrin in whole blood or plasma clots.

The initial prothrombin concentration was at the lower limit of normal and dropped to 40 per cent of normal during the ensuing 3 weeks. This possibly was due to continued blood loss and to specific dietary deficiency. It rapidly returned to normal following the administration of synthetic vitamin K. The fluctuations in the prothrombin concentration appeared to bear no relationship to the prolonged coagulation time and, furthermore, the prothrombin concentration was at no time low enough to account for the delayed coagulation of the blood.[34-39]

TABLE III

Coagulation Times of the Blood of W. J. F. Following the Addition of Protamine

Added protamine solution	Coagulation time of 2 cc. blood specimens of W. J. F.'s blood at 37°C.
(*mg.**)	(*minutes*)
0.00	21
0.001	22
0.005	27
0.010	31.5
0.050	52.5
0.100	120

*Each concentration made up in 0.1 cc. of 0.85% NaCl solutions.

Coagulation defects due to the circulation of anticoagulant substances in the blood are rare. A moderately prolonged coagulation time, presumably due to retention of toxic substances by the diseased kidneys, is sometimes found in severe uremia. In this patient there was no evidence of renal failure and the concentration of nonprotein nitrogen of the blood was normal.

The coagulation time can be markedly prolonged by the intravenous administration of heparin, and the anticoagulant effect of heparin can be neutralized by the addition of protamine to the blood.[40,41] The possibility that the coagulation defect in this patient might be due to an excess of heparin in the blood was eliminated by the addition of protamine to his blood *in vitro* (Table III). The prolonged coagulation time was not shortened by this procedure.

A prolonged coagulation time due to an unidentified circulating anticoagulant was recently reported by Lozner, Jolliffe and Taylor.[42] The plasma of their patient produced a marked prolongation of the coagulation time of normal blood when added in ratios of 1:10 and 1:20. In our patient no significant anticoagulant effect could be demonstrated by similar tests (Table IV). However, these experiments do not completely exclude the possibility of the presence of a circulating anticoagulant. If such an anticoagulant were present in a relatively weak concentration, its effect might remain undetected by the technic employed.

TABLE IV

Coagulation Times of the Blood of a Normal Subject Following the Addition of Graded Quantities of the Plasma of W. J. F.

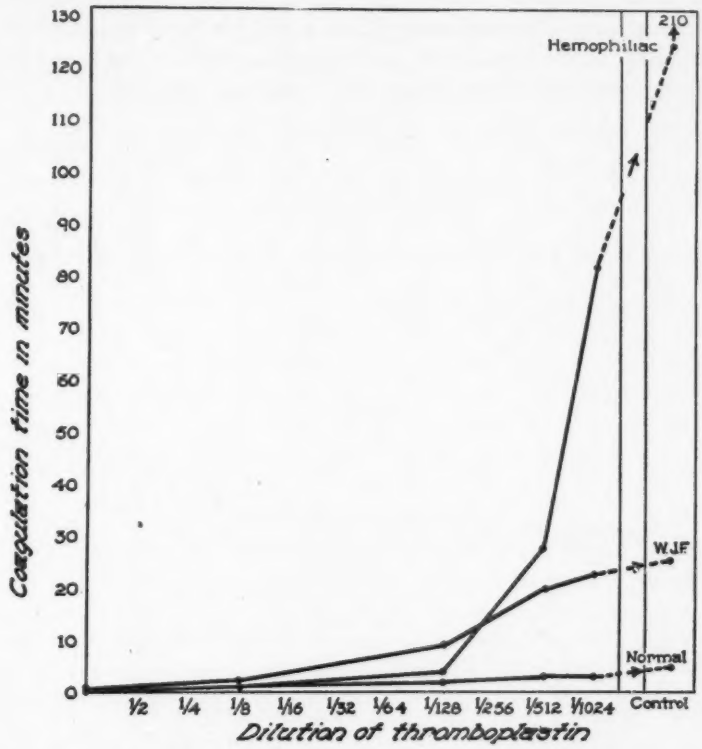
Added citrated plasma of W. J. F.	Coagulation time of 2 cc. specimens of normal blood at 37°C.
(*cc.*)	(*minutes*)
0	8.5
0.02	10
0.05	9
0.10	11.5
0.20	11

The possibility of the presence of both thrombocytopenic purpura and hereditary hemophilia in this patient seemed remote since his clinical history was not that of hemophilia and his family history gave no support to such a diagnosis. The question of an acquired abnormality similar to that of hemophilia was considered. The exact nature of the hemophilic defect is disputed. Quick [43] has recently defended the traditional hypothesis that it is a pure thromboplastin deficiency caused by decreased rate of lysis of platelets. This view is perhaps supported by the observation that the prolonged coagulation time of hemophilic blood can be shortened to normal either by the addition of a minute quantity of tissue extract containing thromboplastin, or by the addition of a small amount of normal blood or plasma. On the other hand, the group working at the Thorndike Memorial Laboratory believe that the platelets are normal in hemophilia and that the defect in coagulation is due to the absence of an activator which is present in the globulin fraction of normal blood.[44–47]

We performed a number of experiments to determine if a coagulation defect of the blood similar to that of hemophilia was present in this patient. In Text-Figure 1 are given the results of experiments which were devised to compare the coagulation times of the *whole blood* of the patient with those of a known hemophiliac * after the addi-

* The hemophiliac was patient L. T., whose case has been described in detail.[40]

tion of progressively decreasing concentrations of thromboplastin solution.* A similar series of tests, the results of which are given in Text-Figure 2, was performed in order to compare the *plasma* coagulation times of the patient with those of a hemophiliac after the addition of thromboplastin solutions.†



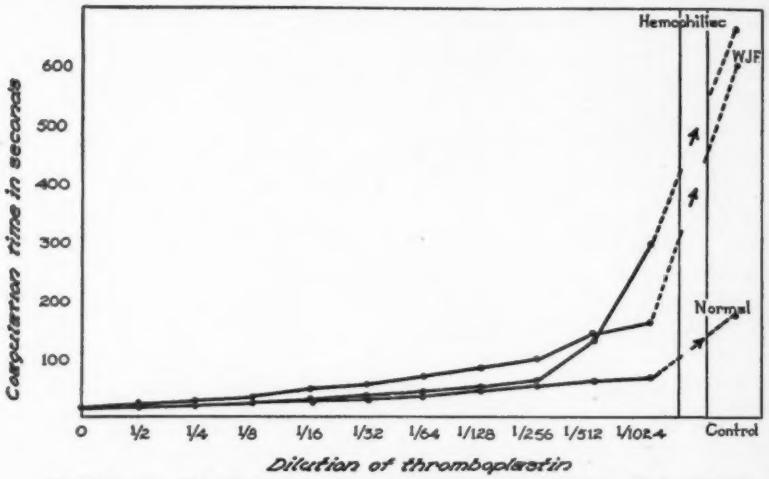
Text-Figure 1. The effect of progressively decreasing concentrations of thromboplastin solution on the coagulation time of the whole blood of a normal subject, a known hemophiliac, and of the patient W. J. F.

In full strength or in moderate dilution, the thromboplastin solution caused a greater reduction in the coagulation time of the hemophilic blood than of the patient's blood. With greater dilution of the thromboplastin solution there was less effect on the coagulation time of either

* The thromboplastin solution was prepared as for the prothrombin test according to the method of Quick.[42] The tests were performed at 37° C. in 12 by 100 mm. tubes. 0.1 cc. of progressive dilutions of the thromboplastin solution was added to 2 cc. specimens of blood. The tubes were inverted once for mixing.

† The tests were performed in the same manner as the Quick prothrombin test [42] except that a series of dilutions of the thromboplastin solution was used for testing.

of the bloods and they approached the control time in which physiologic saline solution was substituted for thromboplastin. Similar results were obtained in the tests performed on the respective plasmas. The lessened coagulative activity of the concentrated thromboplastin solutions on the patient's blood or plasma was probably due to the slightly diminished prothrombin concentration in his blood. On the basis of their reactions to thromboplastin, one would not be able to distinguish between the coagulation defect of this patient and that of a hemophiliac with a similar degree of hypoprothrombinemia. However, in another



Text-Figure 2. The effect of progressively decreasing concentrations of thromboplastin solution on the coagulation time of recalcified plasma of a normal subject, a known hemophiliac, and of the patient W. J. F.

series of experiments in which the coagulation times of the whole blood of the patient were compared to those of the hemophiliac after the addition of graded quantities of normal blood (Table V) and plasma (Table VI), a difference between the two bloods was clearly demonstrated. The same quantities of normal blood or plasma which produced a marked reduction in the coagulation time of hemophilic blood had little effect on the coagulation time of the patient's blood. Furthermore, the transfusion of whole blood did not shorten the coagulation time of the patient's blood in a manner comparable to that usually seen in hemophilia.

Unfortunately, with the present available methods, it is impossible to measure the thromboplastin content of human blood, and therefore one cannot prove that a condition of hypothromboplastinemia exists. It seems reasonable to assume that it is present, however, when, as in

the present case, (1) there is a significant degree of thrombocytopenia, (2) the structure of the platelets is altered, (3) the coagulation defect is corrected by the addition of thromboplastin, and (4) all other known causes of delayed blood coagulation have been excluded.

TABLE V

Coagulation Times of the Blood of W. J. F. and of a Known Hemophiliac before and after the Addition of Graded Quantities of Normal Human Blood

Added citrated normal blood	Coagulation time of 2 cc. blood specimens at 37°C.	
	Hemophiliac	W. J. F.
(*cc.*)	(*minutes*)	(*minutes*)
0 (control)	120	23.5
0.02	24	17
0.05	19.5	16
0.10	13	19
0.20	11	20

If it is assumed that the cause of the delayed coagulation time in this patient was hypothromboplastinemia due to deficient platelet numbers and function, then the results of these experiments would tend to support the belief of the Thorndike group that the substance in normal blood which is effective in shortening the coagulation time of hemophilic blood is not thromboplastin but some other plasma constituent.

The Thrombotic Features

Thrombosis may be caused by alteration in the diameter of the vessel lumen with consequent changes in the blood current, by damage to the intima of blood vessels, and by physicochemical changes in the blood. Alterations in the blood itself, which, under certain circumstances, may contribute to the formation of thrombi, include increases in the number or agglutinating power of the platelets, the coagulability of the blood, the quantity of the globulin-fibrinogen fraction of the blood proteins, and increased liberation of thromboplastin from disintegrated blood and tissue cells.

TABLE VI

Coagulation Times of the Blood of W. J. F. and of a Known Hemophiliac before and after the Addition of Graded Quantities of Normal Human Plasma

Added citrated normal human plasma	Coagulation time of 2 cc. blood specimens at 37°C.	
	Hemophiliac	W. J. F.
(*cc.*)	(*minutes*)	(*minutes*)
0.0 control	120	25
0.02	25	19
0.05	15	20.5
0.10	14.5	22
0.20	15	25

Despite the presence of a marked hemorrhagic tendency, the patient developed thrombosis in the cavernous and basilar sinuses, in the veins of the arms, and in the laryngeal submucosa. It is difficult to determine the cause of these thrombotic episodes. Because of the marked bleeding tendency, it seems logical to assume that spontaneous hemorrhages into the septa of the cavernous sinuses resulted in narrowing of the lumina, producing endothelial damage with consequent thrombosis. The partial thrombosis of the basilar sinuses may have been caused by propagation from the cavernous sinuses. On the other hand, the original thrombosis may have occurred in the nasal tract following treatment of epistaxis, and may have been propagated thence to the cavernous sinuses.

The thrombi noted in the veins of the submucosa of the larynx were probably secondary to a large hemorrhage which had occurred several weeks before death. Phlebothrombosis in the arms occurred only along the course of veins traumatized by venipuncture. No primary alteration in the endothelium of arteries or veins could be detected in any of the material studied.

Although the primary cause of thrombosis is not discoverable in this case, the laboratory data give evidence that the blood platelets failed to function properly in the process of blood clot retraction; [48,49] they failed to assist in maintaining capillary continuity; [5] and they did not supply enough thromboplastin to ensure an efficient coagulation of the blood.

Summary

A case of primary thrombocytopenic purpura is presented, in which there was a markedly prolonged coagulation time of the blood in addition to the usual findings of prolonged bleeding time, diminished blood clot retraction, and increased capillary fragility. The patient had had frequent epistaxis and had bruised easily since childhood, and for 1 year before death these symptoms had increased in frequency and severity and were associated with the appearance of numerous petechial hemorrhages on the lower extremities. While in the hospital the patient suffered from a generalized purpuric eruption, epistaxis, retinal hemorrhages, bleeding into the oral and pharyngeal mucous membranes, bleeding hemorrhoids, bleeding into the right maxillary sinus, subpleural hemorrhages, a large submucous hemorrhage into the posterior wall of the pharynx, gross hematuria, and a large meningeal hemorrhage. Terminally, he presented the signs of bilateral cavernous sinus thrombosis.

The post-mortem examination disclosed: (1) Hemorrhages in the subarachnoid space; in the fibrous septa, nerves and ganglia of the

cavernous sinuses; and in the mediastinum, lungs, subpleural space, right auricle, kidneys, stomach, adrenal gland, vocal cord, scalp, and skin. (2) Thrombosis of the basilar and cavernous sinuses, and of veins in the arms and laryngeal submucosa. (3) Generalized arteriosclerosis with atherosclerosis of coronary arteries and aorta, and hyperplastic arteriosclerosis involving small arteries of the myocardium, lungs, gallbladder, and of the capsules of the pituitary and adrenal glands.

The prolonged coagulation time was thought to be due to hypothromboplastinemia caused by deficient platelet numbers and function because: (1) there was a significant degree of thrombocytopenia, (2) the structure of the platelets was altered, (3) the coagulation defect was corrected by the addition of thromboplastin, and (4) all other known causes of delayed blood coagulation were eliminated. The coagulation defect differed from that found in hemophilia in that it was not corrected by the addition of small amounts of normal blood or plasma *in vitro* nor by the transfusion of whole blood *in vivo*.

The cavernous sinus thrombosis appeared to be due either to propagation of a thrombus from the nasal submucosa, or to spontaneous hemorrhages into the septa of the cavernous sinuses with resulting endothelial damage and narrowing of the lumina.

We wish to thank Joan Howard Hudson for the statistical analyses.

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[*Illustrations follow*]

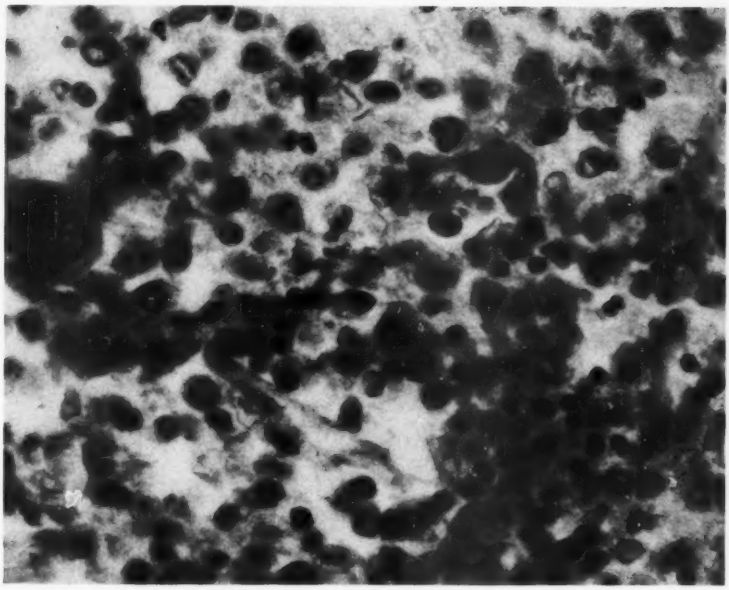
DESCRIPTION OF PLATES

PLATE 239

FIG. 1. Bone marrow of lumbar spine. × 650.

FIG. 2. Cavernous sinus showing thrombosis with organization. × 120.

1



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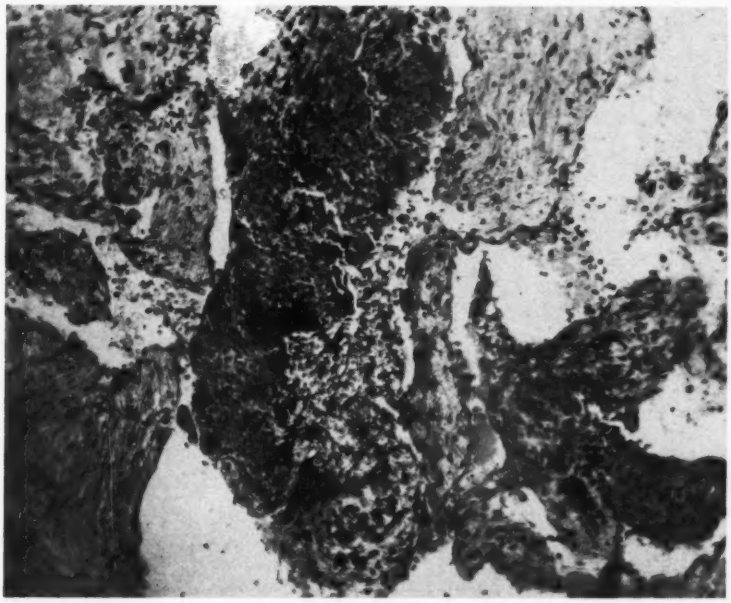
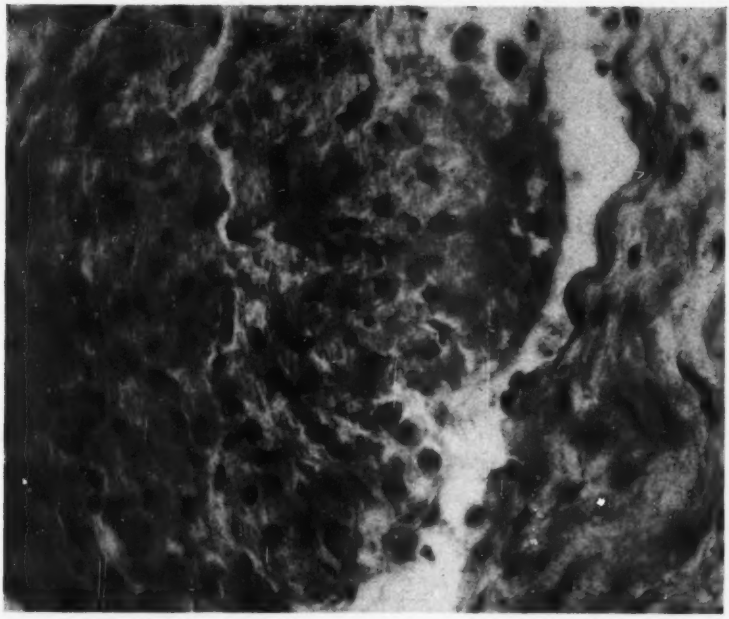


PLATE 240

FIG. 3. Higher magnification of one area shown in Figure 2. Late organization of the thrombus. × 500.

FIG. 4. Portion of thrombus from cavernous sinus showing occasional leukocytes and erythrocytes, separated by granular masses of platelets. × 750.



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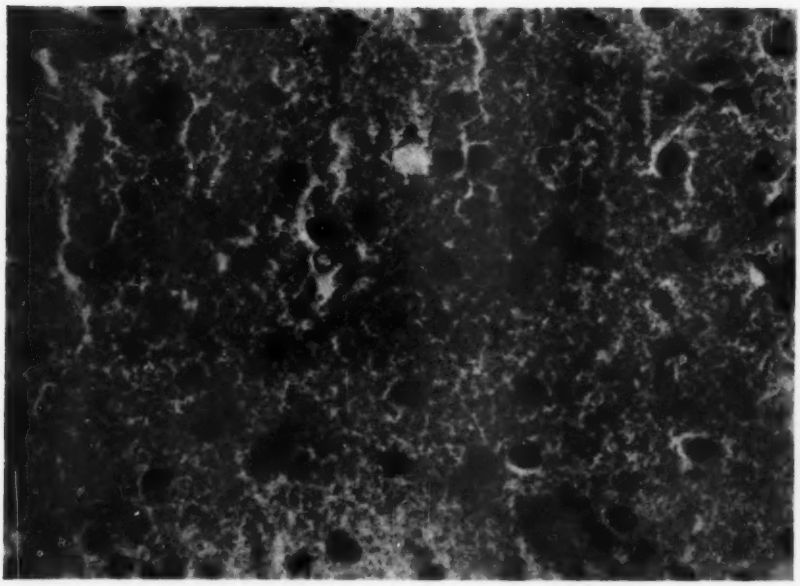

4

STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

VIII. Experimental Morphologic Pathology of the Chemical Transmitter of Nerve Impulses in the Course of Wallerian Degeneration *

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The effects of nerve impulses on the structure of muscle are unknown. The morphologic effects of wallerian degeneration, revealed by the gold-and-teasing method of whole muscle fibers, upon the voluntary neuromuscular apparatus, are likewise practically unknown. It is emphasized at the outset of this paper that the value of the observations to be presented depends largely upon the histologic method selected to demonstrate the structural union of nerve and muscle. Objective evidence of the morphologic transmitters of nerve impulses has not been conclusively demonstrated. Presumptive evidence has been presented that the motor end-plates influence the structure [1] of, and that they discharge granules of acetylcholine [2] into, the myoplasm of striated muscle during the early stages of poliomyelitis, certain other acute infections, rigor mortis, injection of lactic acid locally into the muscle, shock, and specific drug actions. There appears to be a metabolic destruction of the motor end-plates under certain nosologic conditions.

The whole theory of the chemical transmission of nerve impulses was the result of a simple and classical experiment by Loewi [3] on the heart of the frog. He established that, upon stimulation of the vagus nerve, acetylcholine appeared in Ringer's solution placed within the cavity of the heart. When this fluid from the donor heart was placed in the cavity of the denervated recipient heart, the inhibition was comparable to that of vagus stimulation. The accelerator nerves were stimulated, and evidence was obtained that an accelerator substance, adrenalin, appeared in the perfusion fluid. Loewi established the fact that a nerve impulse is transmitted to cardiac tissues indirectly by chemical substances which were called chemical transmitters.

When normal blood is circulating through the heart there is rapid disappearance of acetylcholine. This was proved to be due to a hydro-

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lyzing enzyme in the blood named cholinesterase. This enzyme splits acetylcholine and renders it ineffective. It was likewise proved by Loewi [3] that eserine has an inhibitory effect on cholinesterase. That eserine prolonged the effect of vagus stimulation had been known for a long time.

Dale, Feldberg, and Vogt,[4] after indirect stimulation through the nerve fibers of perfused voluntary muscles, found acetylcholine in the eserinized perfusion fluid. They stated that artificial perfusion with an eserine-containing saline fluid was necessary for success and that all that their evidence showed was that acetylcholine, which had escaped from the sites of its liberation, required protection by eserine to enable it to diffuse into fluid perfused through the vessels. Curare was demonstrated to have an effect on the receptive element by making the muscle substance more resistant to the action of acetylcholine. They likewise established the fact that direct stimulation of muscle tissue 10 days after denervation did not release a trace of acetylcholine. This substance, therefore, was released from the nerve endings and was not a product of muscle activity or of the blood supply. Brown, Dale, and Feldberg [5] then demonstrated that acetylcholine injected into the artery close to the bled muscle caused a rapid muscle twitch similar to that produced by nervous stimulation.

Nachmansohn [6] demonstrated subsequently that cholinesterase was more highly concentrated about the nerve endings in the sartorius muscle of the frog than at the ends of the muscle. Nachmansohn and John [7] found choline acetylase in the nerve axon, and stated that the presence of this enzyme is consistent with the view that the primary event responsible for the alteration of the axon membrane during the passage of the nerve impulse is the release and removal of acetylcholine, and that the energy-rich phosphate bonds during recovery are used for the synthesis of the acetylcholine removed during activity. It was found that only a part of the initial enzyme activity had been lost 3 days after nerve section when conductivity had disappeared.

The relationship of the production of acetylcholine and its destruction to the pleomorphism of the motor end-plates and the various fiber types has not been established. It is known that differences among the fibers disappear after starvation, denervation, muscle contraction, and heat rigor. The mechanism of production of dark and light fibers in the same mammalian muscle is still obscure.

Waller [8] was the brilliant initiator of the studies in the degeneration of nerves almost 100 years ago, yet wallerian degeneration of the neuromuscular apparatus in voluntary muscle has never been followed in a closely graded series of changes by the gold-and-teasing method of whole muscle fibers following the sectioning of the motor nerves. Waller

made his observations primarily on fresh tissue and on tissue treated with caustic potash. Huber [9] used methylene blue in his experimental studies and cited the limitations of this method. Cajal [10] used the silver and sectioning method which likewise has its limitations. The advantages and disadvantages of the gold method will be cited under "Materials and Methods" in this paper.

The precocious failure of transmission of nerve impulses after the third day in the course of wallerian degeneration of cholinergic nerve fibers has been attributed recently (a) to an early degeneration of nerve endings (Titeca [11]); (b) to a decrease of acetylcholine liberation (Coppée and Bacq [12]); (c) to heterochronism (Chauchard [13]); and (d) to a failure of conduction in the nerve fibers (Rogers and Parrack [14]). The contradictions of fact and of inference involved in these four interpretations prompted a restudy of the problem by Lissák, Dempsey, and Rosenblueth,[15] who came to the conclusion that the mechanism of liberation of acetylcholine at cholinergic nerve endings upon the arrival of a nerve impulse is unknown and that the failure of transmission of nerve impulses about the third day after denervation is due to a progressive decrease in acetylcholine liberation, thus substantiating the theory of Coppée and Bacq. Lissák, Dempsey, and Rosenblueth concluded that total failure of transmission about the third day after nerve section was only a terminal step in a gradual decaying process and that this failure of transmission preceded the sudden failure of conduction in the distal stump of the cut nerve.

One great gap in our knowledge of the transmission of nerve impulses through motor end-plates is due to the absence of conclusive evidence based on experimental morphology. The chemical and physiologic actions in the body are intimately related to the structural arrangement of the protoplasm. If the motor end-plates are microscopic endocrine glands that discharge, under exaggerated stimulation, neurogenic bodies or neurosomes composed of acetylcholine, then microscopic morphology must substantiate this claim.

In this paper we have occupied ourselves with the exact and clear description of those morphologic facts which are certain and easily verified. This has necessitated a large number of illustrations in the form of untouched photomicrographs made from unimpeachable preparations. This gives an atlas of the sequence of changes during the course of wallerian degeneration of the motor end-plates which may serve as a source of reference and a line of departure for additional experimental studies and for the basis of the hypothesis that ameboid motion occurs at these motor end-plates and that a granular secretion is discharged into the muscle.

The objectives of this paper, therefore, are the morphologic demon-

stration of untouched photomicrographs which substantiate the following statements: (1) that there is a morphologic as well as a chemical transmitter of normal and abnormal nerve impulses; (2) that the motor end-plates under normal conditions discharge periodically a finely granular secretion which forms a nebulous rhythmic wave of diffusion into the myoplasm, and which may be agglutinated into large neurosomes in the course of wallerian degeneration; (3) that these motor end-plates undergo a periodic retraction and expansion by ameboid motion in relation to the storage in them, and the discharge from them into the myoplasm, of neurogenic substances; (4) that the failure of transmission of nerve impulses into the muscle about the third day after nerve section parallels the disappearance of the discharge and diffusion of the fine neurogenic granular secretion and the appearance of pathologic changes in the neuromuscular apparatus; (5) that the functional, dark type of muscle fiber, which progressively disappears after denervation, is normally associated with the periodic discharge and diffusion of the fine neurogenic granules of acetylcholine from the motor end-plate into the myoplasm at the onset of contraction; (6) that the same muscle fiber may be either dark and granular or light and relatively agranular dependent upon the functional phases of either acetylcholine diffusion, at the onset of contraction, or acetylcholine destruction by cholinesterase during full contraction, respectively, when the fibers were fixed during fractional contraction; (7) that the increase of the granules around the bloated subsarcolemmal nuclei is coincident in time with the persistence of cholinesterase after denervation; (8) that the facts support the claim that denervation atrophy of muscle is associated with the loss of the normal periodic diffusion from the motor nerve endings into myoplasm of acetylcholine granules having a strong affinity for gold, and that, conversely, the hypertrophy of normal muscle due to the chronic effect of exercise may be the result of the quantitative increase, over a unit of time, of the discharge of neurogenic granules from nerve endings to muscle; and (9) that the relationship of nerve to muscle is one of periodic anatomic continuity through the confluence and compounding of neurogenic and myogenic substances. The structure of voluntary muscle, therefore, has a dual composition.

Materials and Methods

One hundred adult white rats *(Mus norvegicus)*, 10 to 12 months old, with an average weight of 255 gm., were used. All surgical procedures were carried out under ether anesthesia and aseptic precautions. Segments 1 to 3 cm. in length were excised from the right sciatic nerve at the level of the trochanter. The left sciatic nerve remained intact and the neuromuscular apparatus of the left gastrocne-

mius muscle was used as a control. Since our observations were confined largely to the first 30 days after section of the right sciatic nerve, our experiments were not complicated by regeneration, as was proved by lack of response of the muscle to electric stimulation of the distal stump of the nerve. The reaction of the gastrocnemius-soleus muscle to indirect faradic stimulation was tested through the distal stump of the cut sciatic nerve immediately after the operation and on the dates selected for excision of the muscle. There was progressive decrease in the response of the muscle to stimulation of the nerve until the third to the fifth day, after which nerve stimulation was ineffective in producing muscle contraction.

In preparing the muscle for the gold technic previously described,[1] the animals were placed under light ether anesthesia and the gastrocnemius muscles quickly excised. This procedure avoided the changes that might occur with post-mortem rigidity.

At designated times after nerve section some animals were curarized, by either intramuscular or intraperitoneal injection of approximately 1 mg. per kg. of d-tubocurarine chloride (Squibb), until neuromuscular block occurred. Under light ether anesthesia the sciatic nerve and gastrocnemius muscle were exposed on the left and right side. In some, the sciatic nerves on both sides were stimulated at the rate of 5 to 10 times per second for 30 seconds with the double electrodes composed of nickel-plated copper, of the Dumont variable frequency stimulator type 210.

After the operation, the gastrocnemius muscles from 5 rats were excised after each 24-hour interval up to 15 days. The muscles from 2 rats were excised daily from the 15th to the 25th day after nerve section, and those from the remaining 5 rats were excised on the 30th day.

The excised muscles were subjected to various histologic methods for the identification of lipoids and other substances and to visualize the nuclear and cytoplasmic constituents of the neuromuscular apparatus. The Cajal [10] silver method, as well as that of Bielschowsky modified by Boeke,[16] is a good one to identify nuclei, neurofibrils, and the perterminal network of Boeke. Since sectioning obscures the whole structure of the neuromuscular apparatus, and since the chemicals used are found to alter the real structure of the union of nerve and muscle, the silver method should be checked against results obtained by methylene blue and gold. Murray [17] stated that the heavy treatment of tissues with formalin and other chemicals in the silver method is bound to cause serious shrinkage effects and that the process is of such capricious character that tissues treated in exactly the same way show different results.

Huber,[9] in his observations on the degeneration and regeneration of

motor and sensory nerve endings in voluntary muscle, used methylene blue. He stated that, due to limitations of methylene blue, the changes in the nerve endings were not revealed beyond the second day after nerve section, and that the precariousness of the method is such that in normal tissue nerve fibers and nerve endings remain now and again wholly unstained or are only partially brought to view.

The inconstancy of Ranvier's gold method is pointed out by Galigher [18] as follows:

> "Unfortunately the formation of gold deposits upon the structures is brought about by an exceedingly delicate reaction which is not well understood, and cannot be obtained with any degree of certainty. The method is notoriously unreliable, and several trials must often be made before a satisfactory impregnation is obtained. However, the results are sufficiently beautiful to justify the effort required to obtain them. It is regrettably true that for nearly fifty years no effort has been made to improve the method in this respect."

We [1,2] have succeeded in obtaining consistent results with our modification of the gold method. Since gold chloride forms *in vitro* periodic precipitates of the Liesegang type with acetylcholine, choline, lecithin, cholesterol, and certain other lipoidal substances, comparable to those cross striations of capillary chemistry previously published,[1] we are inclined to attribute a specific reaction between gold and the normal and abnormal axonic transmitters. The axonic substance is the part that dominantly reacts to gold, but under certain conditions the products of metabolism or breakdown of myelin likewise combine with gold. In the chemical detection and quantitative analysis of acetylcholine and choline by Loach,[19] gold chloride is used. The resulting compound is either acetylcholine aurichloride or choline aurichloride.

The striking neuromuscular changes demonstrated by the gold-and-teasing method will constitute the anatomic basis of this report. The one limitation is the lack of clear visibility of the nuclei. This may be revealed by the observation of neighboring tissues with nuclear stains and with silver. By the use of multiple neurologic methods, confidence is gained regarding the true structure of the union of nerve and muscle, and of the advantages of each method.

The experimental and control muscles were run through the identical fluids for the same periods of time.

The detection of the granular and agranular muscle fibers was also made by the study of fresh tissue in physiologic salt solution at 37°C., according to the method of Denny-Brown [20] and Hines.[21]

The success of our modification of the gold-and-teasing method for whole muscle fibers appeared to be due to the following factors: (1) the initial acidulation of the finely cut fresh muscle with lemon juice or

citric acid, which fixed either acetylcholine or choline: the strong bases, choline and acetylcholine, are stabilized by this initial treatment with acid; (2) the gold chloride, which appears to have a selective chemical affinity for the normal axon and its secretory discharge into muscle, and for certain abnormal products in the myelin; (3) the reduction of the gold by the use of formic acid, which stabilized the normal and abnormal nerve products discharged by the hypolemmal axons into the denervated myoplasm; and (4) the retention of the anatomic continuity of the relationship of nerve and muscle by the teasing of whole muscle fibers and nerves. The continuity of long stretches of the epilemmal axon, hypolemmal axon, sarcolemma, the granules of the sole plate of Kühne, and the cross striations of the muscle fiber are preserved and may be studied in one field of microscopic observation. This relationship is obscured by sectioning muscle fibers after silver impregnation. The granules of Kühne are not stained by methylene blue. Dark muscle fibers have a strong affinity for gold and are dark red, purple, or blue: in the light fibers these colors are decreased or absent in teased specimens.

Results: Experimental Morphology

1. *The Pleomorphism of the Normal Motor End-Plates in Relation to Dark and Light Voluntary Muscle Fibers*

The dark and light muscle fibers were clearly evident in the normal control gastrocnemius muscles after the gold technic (Figs. 1, 10, 22, 34, and 35). The dark muscle fibers had more material with an affinity for gold than the light muscle fibers. The dark anisotropic, transverse bands in the dark muscle fibers were usually broader and darker than those in the light fibers. On the other hand, the light, isotropic, transverse striations in the light muscle fibers were usually not only broader than the corresponding light spaces in the dark muscle fibers, but there was less material that had an affinity for gold in the light spaces of the light muscle fibers than in those of the dark fibers.

The width of the muscle fiber was a variable factor depending upon fixation in a state of either isometric and isotonic contraction or the termination of relaxation at the onset of contraction. Some light muscle fibers, therefore, were smaller in diameter than the dark ones. The variable capacity of the different muscle fibers for gold impregnation was just as reliable as, and more permanent than, the study of fresh teased muscle. The dark muscle fibers may be classified as hyperchrysophilous and the light ones as hypochrysophilous. The cross striations

in dark fibers are composed of neurogenic and myogenic substances. There were multiple gradations in the affinity of the fibers for gold between the two extremes. The fading of the striations in denervated muscle produced fibers that may be classified as achrysophilous.

The motor end-plates in the dark and granular muscle fibers usually possessed coarse fronds or knob-like terminals. Some of these fronds were surrounded by a light halo-like space. The retracted motor end-plates were usually surrounded by more dark granular material of the sole plate of Kühne than those of the relatively expanded end-plates in the light muscle fibers. There appeared to be a direct continuity in some places between the granules of the sole plate of Kühne and those condensed in the periodic dark cross striations of the myoplasm.

The granules of the sole plate of Kühne appeared to be derived from two sources; namely, from the granular transformation and permeability of the terminals of the hypolemmal axons of the motor end-plate, and from the nuclei of the sole plate. This granular material of the sole plate normally appeared to diffuse in a periodic manner throughout the myoplasm of the dark muscle fiber. The structure of the motor end-plate in the light muscle fiber was usually one in which there was an ameboid expansion and an attenuation of the hypolemmal axons of the motor end-plate. There was, likewise, a decreased amount of the diffusible granules of the sole plate of Kühne around these relatively expanded motor end-plates of the light muscle fiber. Because the dark muscle fiber had a strong affinity for gold and this quality was gradually lost by denervation, it was compared to the stage of periodic discharge and diffusion of acetylcholine from the motor end-plate into the myoplasm. The dark muscle fiber was correlated with the termination of relaxation, or the onset of muscle contraction, and the light one with the phase of active, full contraction, in the fractional contraction of the muscle as a whole. This fractional contraction is lost progressively by denervation and its loss is correlated with the loss of the phase of differential diffusion of acetylcholine into the muscle. It has been practically impossible to catch a muscle fiber in the state of completely unstable and physiologic relaxation by any histologic technic used to date. The dark muscle fiber probably represents the structure nearest to that of relaxation, or the onset of contraction of the myoplasm produced by the periodic diffusion of acetylcholine.

The decreased visibility of the cross striations at the onset of contraction, observed in living muscles by many investigators, may represent this stage of the periodic discharge and diffusion of acetylcholine. The periodic structures in the muscle fiber would then be obscured, altered, and realigned in a rhythmic manner by the capillary chemical changes in the metabolism of the neuromyoplasm. The shuttle-like

shift of the cross striations described by Jordan [22] could easily be explained on the above basis.

2. The Progressive Loss of the Dark Voluntary Muscle Fibers after Denervation

There was a progressive loss of muscle fibers of the dark type beginning 24 to 48 hours after denervation. There was an agglutination of the fine granular material that had an affinity for gold into coarse clumps, which gave a flaky appearance to the myoplasm of the muscle fiber (Figs. 6 and 7). This was a histologic sign of the beginning loss of the normal nebulous diffusion in a periodic manner of the granular substance discharged from the denervated motor end-plate. This was microscopic evidence of the beginning dissociation of the nerve and muscle substances in the muscle fiber, characterized by the initial changes in the segregation and accumulation into larger aggregates of the normally fine neurogenic granules. It was a sign of the beginning loss of substantial influence of the innervation upon the muscle. A search is now in progress to produce the chemical denervation of muscle by the injection of some substance that will combine with the chemical transmitter of nerve impulses and thereby inactivate, through segregation, the neurogenic from the myogenic substances in the muscle fiber. Preliminary experiments point to the fact that DDT can play this rôle.

There was also histologic evidence of the loss of the normal fractional contraction of the muscle fibers after denervation. All of the motor end-plates in a specific field were either abnormally retracted with an increased affinity for gold, or were expanded and decreased in their capacity to take the gold (Figs. 4 to 9). On the third day, there were certain fields within the denervated muscle that were totally devoid of muscle fibers of the dark type (Figs. 2 and 11). From the fifth to the tenth day (Figs. 12 to 16) practically all of the dark type had progressively disappeared. The dark muscle fiber had coarse, irregular, longitudinal myofibrillae, whereas those in the light muscle fiber were smaller in diameter and more regularly arranged. The arrangement of the myofibrillae appeared to be correlated with the diffusion and disappearance of the fine granular material that had an affinity for gold. The accumulation of this granular material characterized the dark muscle fiber that was usually narrow and coarsely striated in a longitudinal direction, whereas the light muscle fiber, characterized by the disappearance of the granules with an affinity for gold, was usually wide and composed of fine fibrils arranged longitudinally. The accumulation and depletion of the chrysophilous granules appeared to be correlated with the functional activities of the muscle fiber, based on the diffusion and hydrolysis of the granules of acetylcholine.

3. *The Progressive Pathologic Changes of the Motor End-Plates after Denervation*

During the first 48 hours (Figs. 4 to 9, and 36 to 49) after nerve section, there were relatively normal motor end-plates scattered among those undergoing structural changes. There were noticeable structural changes of the end-plates and adjacent epilemmal axons within the first 24 hours. These were detected by beginning enlargement of about 10 per cent of the epilemmal axons and the formation of retention cysts on the ramifications of some retracted hypolemmal axons of the motor end-plates (Figs. 5, 36, and 37). This retraction, increased affinity for gold, and cystic enlargement of the branches of the nerve endings became structural characteristics in certain microscopic fields from 24 to 48 hours after nerve section (Figs. 6, 9, 41, and 42). Around some retracted nerve endings there was evidence of the granular sole of Kühne, whereas these granules were absent around other retracted nerve endings. The granular sole was most frequently absent around the expanded endings composed of elongated and attenuated branches (Figs. 7, 40, and 45).

In other fields the nerve endings were expanded, some had decreased affinity for gold, and their ramifications were exceedingly attenuated (Figs. 40, 45, and 49). This uniformity of retraction of the nerve endings on certain trees of innervation, and the expansion of all of the nerve endings on others, were histologic evidences of loss of the normal pleomorphism usually found on each tree. They are likewise histologic signs of loss of the normal fractional contraction of the muscle fibers under the influence of normal innervation. This was evidence of uniform overstimulation and overexcitation of the motor units innervating the denervated muscle after nerve section. Between 5 and 10 per cent of the nerve endings were replaced by fine granules at 48 hours. This replacement was found in about 60 per cent of the nerve endings at 72 hours. These figures are based on a differential count of 5,000 motor end-plates.

Concomitant with the structural changes of the motor end-plates, there were associated alterations in the structure of the muscle fiber. Coincident with the loss of the normal periodic diffusion of fine chrysophilous granules transmitted from the motor end-plate, there was seen a coarse flakiness of these granules in the muscle fibers. In some trees of innervation the epilemmal axons were fragmented and had a decreased affinity for gold (Fig. 8), whereas in others there was an increased capacity to take the gold with little evidence of fragmentation (Fig. 9). Since these observations were made in the same muscle and since the technic was standardized by running the normal and abnormal

muscles simultaneously and similarly, this was considered a histologic sign of periodic decrease alternating with increase in the quantity of the gold-impregnated material in the epilemmal axons. There was a definite hyperemia of the intramuscular blood vessels and capillaries (Figs. 8 and 9), demonstrated by the increased diameter of the lumen and the packing of these vessels with red blood cells. In other locations the capillaries were collapsed and devoid of cellular elements. This was comparable to the experimental structural conditions produced by poliomyelitis, traumatic and thermal shock, and by the experimental injection of lactic and other acids locally into the muscle.

The depletion of many of the epilemmal axonic trees of their motor end-plates after the third day subsequent to the degenerative cut of the sciatic nerve was structurally associated with the progressive loss of muscle fibers of the dark type (Fig. 2). There was likewise a distinct fading of the dark anisotropic cross striations due to the loss of granules with an affinity for gold. This was due to the depletion from these striations of fine chrysophilous granules that were normally periodically discharged from the motor end-plates and that diffused into the myoplasm. It was imperative, therefore, that frequent comparisons be made of the normal reaction with gold (Figs. 1, 10, 22, 34, and 35) and the loss of this reaction in denervated muscle.

Although it was true that only the axons of the nerve fibers and their end arborizations were dominantly impregnated with gold, there was no difficulty in differentiating medullated nerve fibers with the faint outline of a sheath and the locations of the nodes of Ranvier from the varicose nonmedullated nerve fibers. In the degenerating medullated nerve fiber, the myelin was impregnated more readily than in the normal one, so that the segments of a degenerated medullated sheath may be made out during the early stages, 48 to 72 hours after nerve section. There was distinct segmentation of the myelin during this time, and the degenerated products fused with those in the central axon. It was clearly evident that the extreme distal end of a severed motor nerve and its motor end-plate degenerate before the remaining portions of the same nerve distal to the point of the degenerative cut. The degenerative changes which follow the segmentation of the myelin and the formation of cystic enlargements on the motor end-plates were clearly followed by the gold-and-teasing method applied to whole muscle fibers. Huber [9] stated definitely that it was impossible for him to follow the changes with the methylene blue technic the second day after the section of the nerve. The granular degeneration was well advanced in the motor end-plates 72 hours after denervation (Figs. 50 to 55).

Sprays of medullated nerve fibers underwent progressive degeneration by the depletion and discharge of their degenerated contents, in a centrifugal direction, from 5 to 30 days after nerve section (Figs. 12 to 21, 56, 57, 69, and 70). The discharged combined axonic and myelin substances had a strong affinity for gold and were accumulated in pleomorphic masses within and around the region of the degenerated motor end-plates. The faint, ghost-like outline of the epilemmal axons depleted of substances that had a strong affinity for gold was in striking contrast to that of the accumulated chrysophilous material discharged in a centrifugal direction into the region of the degenerated motor end-plates. Some of these pathologic masses or neurosomes were found out in the myoplasm of the muscle fiber. The muscle fibers 20 days after the degenerative cut (Fig. 19) had lost the appearance of the normally functioning dark type and were very narrow in comparison with the normal. This anatomic evidence shows conclusively that the histologic dark type of muscle fiber is dependent upon the normal functioning of the nerve supply. This functional dark type of muscle fiber, therefore, appears to be produced by the normal periodic diffusion into the myoplasm of the fine granules of acetylcholine discharged from the motor end-plate.

The axonic material which was discharged in a centrifugal direction 30 days after the degenerative cut (Figs. 20 and 21) was seen in relation to only a few of the degenerated motor end-plates. The locations of the degenerated end-plates were shown dominantly by clusters of sole plate nuclei which were surrounded by the granular material secreted by these nuclei. This granular material had an affinity for gold slightly less than that of the material discharged by the hypolemmal axons. The clusters of nuclei were detected as clear, rounded, or oval spaces surrounded by the dark granules (Fig. 20).

In some places in the same muscle, the myoplasm was aggregated into broad and narrow, dark, transverse bands alternating with light ones (Fig. 21). In the dark bands the cross striations were either exceedingly fine or they were completely absent because of the opacity of the dense aggregation of the myoplasm. The light bands were occupied by cross striations which were wider apart than those in the dark bands. This dark and light transverse banding of the narrow muscle fibers was produced by the slow, irregular fibrillation of the muscle fibers. This incoordinate and ceaseless fibrillation, of slow vermiform activity, of the denervated muscle fibers begins about 72 hours after the degenerative cut of the sciatic nerve. This fibrillation was correlated with the loss of the dark muscle fibers and the loss of the transmission of acetylcholine which, normally, chemically tunes the muscle

fiber to the higher pitch or frequency of fast muscle contractions and to the capacity to respond adequately to high frequency stimulation. The chemical tuning process of muscle was roughly analogous to the mechanical tuning of the strings of a piano or violin for response at a higher frequency than that of strings which are loose and sagging.

4. The Experimental Exaggeration of the Discharge of Large Neurosomes into Denervated Muscle

The progressive degeneration of peripheral nerves, clearly described by Parker,[23] was accelerated by the intraperitoneal injection of d-tubocurarine chloride. In some animals, after the muscle was curarized, the distal segment of the cut sciatic nerve was stimulated for 30 seconds, at the rate of 5 per second, with no response of the muscle. This experimental procedure caused a massive accumulation of degenerated nervous substances discharged into the muscle from 5 to 20 days after section of the nerve (Figs. 3, 23 to 33, and 58 to 68). There was complete depletion of the material in the epilemmal axons and an enormous accumulation of this neurogenic material in and around the degenerating motor end-plate, with periodic discharges of pleomorphic neurosomes into the myoplasm. This exhaustion of the axonic spray of its specific substances with strong affinity for gold, and the massive accumulation of these chrysophilous substances at the degenerating end-plate and in the muscle constitute conclusive evidence that there was a substantial transfer of some substance from the degenerating nerve to the denervated muscle. The absence of the normal dark muscle fibers was related to the loss of the normal periodic nebulous diffusion of the granules of the sole plate of Kühne. The agglutination of either the normal or abnormal transmitter substance in a relatively non-diffusible form gave rise to these morphologic changes under the conditions of the experiment. This demonstration of the pathologic structure of the abnormal transmitter substance was produced by acceleration of the discharge and prevention of the normal diffusion of granules into the myoplasm of the muscle fiber. There was great variation in the structure of this abnormal substance transmitted from nerve to muscle (Figs. 3, 23 to 33, and 54 to 68).

The discharge of the degenerated axonic and myelin materials into the peripheral terminal zone of the degenerated motor end-plates had a pleomorphic arrangement; namely, unipolar (Figs. 58 and 59), bipolar (Fig. 61), and multipolar or completely circumferential (Figs. 60 and 62). The chrysophilous material had been discharged into and around the degenerating motor end-plates in a centrifugal direction from the epilemmal axons and there was unimpeachable evidence that

this gold-impregnated material was initially in direct continuity with the structures that innervate the muscle. In one end-plate (Fig. 59) the discharged neurosome was in direct anatomic continuity with the hypolemmal axon of the motor end-plate. It formed what has been designated by some neuroanatomists as an ultraterminal nonmedullated branch of the motor end-plate, ending in the same muscle fiber in an enlarged spherical or oblong terminal. This ultraterminal branch of the motor end-plate, however, was merely the initial phase of the discharge of materials from the nerve terminal with anatomic continuity still maintained. It was the product of the abnormal discharge of substances by abnormal stimulation from the pathologic motor ending, and not a morphologic ending of specific type as claimed by some observers.

When the narrow, dark, and granular muscle fiber was observed in cross section, it was impossible to differentiate and definitely identify the neurosomes in ordinary histologic preparations. Even with lipoidal stains, it was impossible to identify with certainty acetylcholine and choline neurosomes from particles of lecithin and cholesterol. When gold preparations were imbedded in gelatin and cross sectioned with the freezing microtome, there was a striking variation in the size and staining capacity of the gold-impregnated neurogenic granules. The same normal or abnormal muscle tissue may be teased and photographed in its longitudinal projection and then imbedded, cut in transverse sections, and studied in that plane. There was no doubt, therefore, of the intramuscular location of the normal and abnormal neurosomes. At some degenerated nerve terminals, part of the neurogenic substance may be found between fibers after electrical stimulation of the distal stump of the cut nerve.

5. The Increase of Granules around the Bloated Subsarcolemmal Nuclei after Denervation

The clear, rounded, and oval spaces both within the region of the degenerating end-plate and in the granular cytoplasm of the sole plate of Kühne were occupied by nuclei. The nuclei, close to the degenerating nerve endings (the Telodendrienkern of Boeke), appeared first to enlarge and later to become smaller after nerve section (Figs. 50 and 51), whereas the nuclei in the granular sole (Sohlenkerne of Boeke) and the subsarcolemmal nuclei out in the myoplasm were increased in size, for a considerable period of time, and became surrounded by an increased quantity of granules (Figs. 50 to 53). The bloated subsarcolemmal nuclei were depleted of chromatin and showed loss of nucleoli when specifically stained. These bloated vesicular nuclei became surrounded with a greatly increased quantity of chrysophilous granules concomitant

with the loss of the normal periodic discharge of fine granules from the motor end-plates. The peripheral increase of granulation around the muscle nuclei after denervation was likewise coincident with the unneutralized and persistent activity of cholinesterase found by Nachmansohn and John [7] 3 days after nerve section, when conductivity had disappeared from the axon.

The granular material, that had the capacity to take gold, appeared to be contributed by two sources: the granular transformation of the hypolemmal axons of the motor end-plate, and the nuclei of the sole plate and the subsarcolemmal nuclei of the myoplasm. Pommé and Noël [24] stated that the telesomes or sole plate granules decreased or disappeared in progressive muscular atrophy. Tower [25] stated, however, that these granules had not been studied after experimental denervation. The increased visibility of the nuclei in denervated muscle was probably due to three factors: hypertrophy of the nucleus, accumulation of an increased quantity of granules around the nucleus, and decreased cytoplasm which resulted in crowding together the preexisting nuclei. No evidence was found of nuclear division.

The denervation atrophy caused definite shrinkage of the cytoplasm. Tower [25] defended the opinion that denervation atrophy first attacks the sarcoplasm and at a later date the myofibrillar substance. It was noted, however, that there was no discontinuity of the process of atrophy of the sarcoplasm and the myofibrils that would indicate a shift of the process from one structure to another. Tower concluded, therefore, that the sarcoplasm and myofibrils formed a structural continuum. The chemical evidence indicated that the depletion of substance was in fairly equal proportion for both the sarcoplasm and the myofibrils. There was objective evidence in our experimental study with the gold technic that atrophy of the cytoplasm of the muscle fiber was coincident with failure of discharge of the granules associated with the chemical transmission of the normal nerve impulses. The normal myoplasm, therefore, was proved to be composed of myogenic and neurogenic substances. Atrophy was coincident with the loss of one of these substances, namely, the fine neurogenic granules or neurosomes.

6 The Pleomorphism of the Neurosomes in Denervated Muscle

The discharged neurosomes (Ns., Figs. 71 to 75) found in the myoplasm were pleomorphic and hyperchromatic for gold in comparison with the faded hypochromatic cross striations of the muscle fibers in which they were found. These neurosomes may be fusiform (Figs. 71 and 72), irregularly oblong with one end tapering (Figs. 73 and 74), arrowheaded in shape, or rounded droplets that vary in size (Fig. 75).

The droplets formed either loose series of single drops widely separated or closely related series of 2 to 10 droplets. The droplets in the middle of a series were usually larger than the terminal ones. Such series of droplets formed irregular fusiform structures separated by clear spaces. The large fusiform neurosomes may have either serrated (Fig. 71) or festooned (Fig. 72) edges. The sharp projections arranged like saw teeth around the edges of the neurosomes may or may not be in direct alignment with the dark cross striations of the muscle fiber. These neurosomes undergo a granular dissolution, and the granules become incorporated into the myoplasm, and are then aligned with the cross striations of the muscle fiber. These neurosomes were periodically discharged from the motor end-plates into the muscle fiber. During degeneration they were more persistent, agglutinated, and less diffusible than when produced by the artificial overstimulation of normal muscle or when produced by neurogenic shock.

7. The Experimental Production of Giant Muscle Fibers after Denervation

Giant muscle fibers (Gmf., Fig. 76) were found in a few 10-to-14-day denervated muscles after the intraperitoneal injection of d-tubocurarine chloride followed by electrical stimulation of the distal stump of the cut nerve for a duration of 30 seconds at the rate of 5 per second. The diameter of these fibers was 3 or 4 times that of closely related muscle fibers. They were densely impregnated with gold. This represented an abnormal discharge and accumulation of increased quantities of abnormal axonic material in the fiber. The cross striations were seen only at the edges because of the opacity. There was a definite streamlined effect produced by certain large neurosomes (Ns., Fig. 77) upon the cross striations of the muscle fiber. This was detected by the altered arrangement of the cross striations. Certain large muscle fibers (Fig. 78) were not so densely packed with neurosomes as were the giant muscle fibers (Fig. 76).

In some locations the neurosomes were dense and opaque while in others they were light and cross-striated. The dense neurosomes were gradually replaced by granules which eventually became aligned with the cross striations in the muscle fiber.

8. The Intermittent and Progressive Degeneration of the Distal Stump after Section of the Motor Nerve

The degeneration of the distal stump of the sciatic nerve was tested histologically by the gold-and-teasing method of whole nerve fibers and was proved to be progressive in character and not to take place

simultaneously throughout the whole length of the nerve. The changes in the axis cylinder, medullary sheaths, and motor end-plates were unquestionably initially more advanced at the junction between nerve and muscle than in the same nerve fiber far away from the muscle. There was disappearance of the motor end-plates between the third and fifth days. The degenerated material in the trunk of the nerve advanced, in a progressive manner as well as intermittently, in a centrifugal direction. This, then, resulted in a progressive depletion of the proximal end of the distal stump of the nerve while the terminal of the nerve was discharging the degenerated materials into the muscle. One branch of the sciatic nerve was followed just before its entrance into the gastrocnemius muscle 14 days after sectioning. At this location there was complete depletion of the degenerated axonic and myelin substances that had an affinity for gold (Fig. 79). At a slightly more distal point, the gold-impregnated material was found in variable amounts in axis cylinders which were fragmented into rounded, oval, or fusiform bodies with a strong affinity for gold (Figs. 80 and 81). Just before the nerve gave rise to the branches that directly innervate the muscle, there was again a depletion of the axis cylinders of chrysophilous material (Fig. 82). Only in widely scattered areas were five fusiform bodies found that had an affinity for gold. The epilemmal axons of a cut nerve that innervate the muscle are periodically engorged and depleted of the degenerated and fragmented axonic and myelin materials until structural exhaustion occurs. This rhythmic discharge of degenerated nervous material into the muscle continues, in a progressive and periodic manner and in a centrifugal direction, until most of the degenerated material is discharged centrifugally into the muscle.

Discussion

The Mixture of Red and White Fibers in Voluntary Muscle

No attempt will be made to review the extensive histologic literature accumulated over the past 100 years on red and white muscle and on the controversial subject of the so-called mixture of red and white fibers in the same voluntary muscle. The excellent recent reviews and observations on this topic may be consulted: Tower,[25] Cobb,[26] Needham,[27] Hines,[28] Hinsey,[29] Fulton,[30] Forbes,[31] Creed, Denny-Brown, Eccles, Liddell, and Sherrington,[32] Wilkinson,[33] Roberts.[34]

Cobb [26] makes the following important statements (page 519):

"One must review the *anatomy of muscle* carefully in order to understand the possible effects of the nerve impulse upon it. Even in this field one is surprised to find indefiniteness, partly because the classification of the different types of muscle is inherently difficult on account of intergradations and transitional forms;

partly because the histology of muscle has often been overlooked in the study of its physiology";

and (page 520):

"It must be emphasized here, however, that the state of knowledge concerning the red and white muscle fibres is at present so confused, even in the field of anatomy, that much work must be done before physiological interpretations can be acceptable."

Almost 60 years ago, Grützner,[35] stated that every muscle contains two specific types of fibers, often intimately mixed: one kind narrow and dark, the other broad and light. He thought that the dark color of the narrow fibers was due to numerous granules and that all narrow muscle fibers, whether pigmented or not, corresponded to the red, slowly contracting muscles of the rabbit, and that the large, light, agranular muscle fibers corresponded to the rapidly moving white muscle. Knoll,[36] and Knoll and Hauer [36] designated the cloudy muscle fibers as "sarkoplasmareichen" and the clear ones as "sarkoplasmaarmen" or "fibrillenreichen." Krause [37] believed that the different kinds of muscle fibers seen by Grützner and Knoll were dependent upon differences in age: the narrow granular fiber was assumed to be younger than the wide light fiber. Bonhöffer,[38] however, demonstrated that the proportion and distribution of the two types of muscle fibers were the same whatever the age of the frog.

The skeletal muscle may vary in color, not only from species to species, but in the muscles of the same animal. The differences in color of the white meat of the chicken's breast muscle and the deep red of the pectoral muscle of the pigeon or wild duck is very striking. In 1865, Kühne [39] studied the cream-colored and red muscles of the rabbit by spectroscopic methods. He concluded that the deep red muscles had a higher content of myohemoglobin than the light cream-colored muscles.

The structural and functional significance, however, of the narrow, dark, and granular fibers and the wide, light, and relatively agranular fibers found in both the red and white muscles has not been solved to date. It was demonstrated by Schäffer and Licht,[40] Tower,[41] and others that denervation atrophy abolishes the morphologic distinction between the two types of muscle fibers. The mechanism, however, of abolishing this morphologic difference in the two types of muscle fibers found in so-called mixed muscles by denervation was not revealed.

Tower [41] referred to the "large pale" and "small granular" fiber types as follows (page 123):

"What such characteristics may signify for muscle function, difference in contraction rate, in the ability to store specific lipoidal or protein products, does not

lie within the province of this paper to consider. Yet the problem is of prime importance. For not until the conditions are known which gave rise to clear-cut fiber types in one cat and not in a second; not until the distribution of such diverse fibers within the muscle shall be better understood, will it be possible to evaluate small quantitative changes, the possible result of lesions of the sympathetic."

Tower [41] stated that sympathetic denervation did not alter the proportion, distribution, or constitution of the fiber types in voluntary muscle.

It was an error made years ago, that has led to endless confusion, to compare these two histologic and functional types of fibers with the distinct red and white varieties of muscle. In both red and white muscles there are in each muscle dark granular and light agranular fibers. The functional activity of the neuromuscular apparatus is associated with the release, diffusion, and subsequent destruction of acetylcholine, processes which have not been associated, heretofore, with the dark and light muscle fibers in the fractional contraction in the same muscle. In fact, the probable relationship of the Q and J granules of Holmgren,[42] certain liposomes of Albrecht [43] and Bell,[44] interstitial granules of Kölliker,[45] and fine granular neurosomes in muscle fibers has been proposed only recently by us.[46]

Tower,[47] likewise, surmised that the muscle nuclei and sarcoplasm were in some obscure way dependent upon the influence of the nervous system, as is evident in the following statement (page 25):

"In the extrafusal muscle fiber, the aggregation of nuclei around the motor endplate has always suggested a peculiarly intimate relationship of these to the nervous tissue. But nerve lesion affected not only these nuclei physically associated with the disintegrating nervous tissue, but also all the nuclei the entire length of the fiber. Do all these unconnected and seemingly inactive nuclei form, perhaps with the sarcoplasm, an organization, possibly for conduction within the fiber and for excitation of the contractile mechanism, but an organization dependent on the nervous tissue to a degree incompatible with normal existence after nerve degeneration? This is a most alluring interpretation of the reaction of muscle nuclei, in toto, to denervation."

Denny-Brown [20] stated that slowly contracting fibers were able to store lipoids in the form of liposomes with a resultant granular appearance, while few rapid fibers had this property. Histologic differences were found to disappear on emaciation, but the differences in the speed of contraction remained. Denny-Brown claimed that the dark muscle fiber was related to nutrition. He likewise stated that there were certain light muscle fibers which could not be transformed into the dark granular ones. If the light muscle fibers are the stage of full muscle contraction and hydrolysis of acetylcholine, it would not be surprising to find light fibers even in conditions of overnutrition. Regardless of the method used, a certain number of the granular muscle fibers would

be transformed into the agranular type due to contraction. There would be relatively more fibers of the dark granular type observed in muscles of slow activity in comparison to those with high rate of speed of action, because there would be a correlation between the rate of cycles of diffusion and hydrolysis of acetylcholine granules and that of relaxation and contraction of the muscle fiber. The speed of neuromuscular metabolism would, therefore, tend to determine the relative proportion of fiber types. The irreversible high rate of metabolism to the level of fixation, by heat rigor, is associated with the abnormal overproduction of the light agranular fibers in frog muscle.[1]

The mechanism of production of the atrophy of muscle by denervation has been an obscure problem. This, likewise, applies to the hypertrophy of muscle due to the chronic effects of exercise. Carlson and Johnson [48] (page 369) made the following comments:

"The muscle enlargement with correspondingly greater strength, which is developed by work or training, is a commonly observed phenomenon. There is evidence that the muscle enlargement is not due to any increased number of muscle fibers, as might be suspected, but rather to an increase in size of each fiber. The exact nature of this effect, apparently a growth phenomenon, or the mechanism of its production are not yet known. The effect seems in some way to be related to the influence of nerves and nerve impulses reaching the muscle by way of the efferents. At any rate, if the number of nerve impulses is reduced, as in disuse of the muscle, the muscle fibers become smaller, and the whole muscle shrinks in size and becomes weaker. And, if the efferent nerve of a muscle is destroyed by disease or accident, so that all nerve impulses to the muscle are cut off, the muscle shrinks in size greatly and may entirely disappear and be replaced by fibrous connective tissue, leaving no trace of the former structure."

It is our opinion, supported by experimental morphologic evidence presented in this and other papers, that some light has been shed on the obscure mechanism of the atrophy and hypertrophy of voluntary muscle by the elimination and overproduction, respectively, of a substantial transfer of materials from nerve to muscle. The degree of atrophy and hypertrophy appear to be related to the degree of the decrease and increase of the neurosomes respectively discharged from the motor end-plates into the myoplasm of the muscle fiber. There are unquestionably related vascular changes which are likewise important.

Loewi and Navratil [49] proved that atropine, and Navratil [50] that ergotamine, did not paralyze the respective nerves to the effector organs by showing that after their application nervous excitation is still effective in liberating the transmitters. This was likewise shown to hold for nicotine by Feldberg and Vartiainen,[51] and for curare by Brown and Feldberg [52] and Brinkman and Ruiter.[53] These pharmacologic experiments demonstrate that acetylcholine acts directly upon the voluntary

muscle and that chemical antagonists counteract this effect. The true point of attack of these important drugs is upon the neurogenic component of the compound neuromyoplasm. These drugs alter the structure of the motor end-plates and likewise form agglutinated masses of large neurosomes in the myoplasm. The experimental morphologic findings in this paper support the interpretations of the action of the above drugs on the myoplasm. Dale, Feldberg, and Vogt [4] concluded, likewise, that when transmission of excitation from the nerve to the perfused muscle is prevented by curarine, stimulation of the motor nerve fibers causes the usual release of acetylcholine. The effect of eserine was to aid the diffusion of acetylcholine from the site of its release in the nerve endings of voluntary muscle into the blood vessels. The experiments of Dale and his colleagues demonstrated quite definitely that the appearance of acetylcholine in the perfusion fluid is not directly or indirectly produced by the contraction of the muscle fibers.

The absence of nuclear division in denervated muscle confirms the findings of Willard and Grau.[54] The hypertrophy of the nucleus and the peripheral accumulation of granules confirm the findings of Tower.[47] Our observations confirm the statement of Hines and Knowlton [55] that the rat is a very satisfactory animal upon which to study the changes in skeletal muscle after denervation. They did not, however, study those changes in the neuromuscular apparatus, as reported in our present paper, because of the obliterating effects of the histologic methods employed by them.

Feiss and Cramer,[56] in discussing the nature of wallerian degeneration, stated that the proliferation of the neurilemmal nuclei and the fragmentation of the myelin sheath must be considered separately. The cytologic changes in the degenerating medullated nerves as correlated with the changes in the axon and myelin sheath are problems for future investigation. The changes revealed by the gold technic, however, point to the fact that the traumatic sectioning and resulting continuous overstimulation of the nerve produce an abnormal breakdown and discharge of neurogenic substances that proceed at a more rapid rate than the process of elaboration. The function of the cell body is required for the synthesis of the normal transmitter in the intact nerve fibers. That acetylcholine is formed in cholinergic nerve fibers was established by Loewi and Hellauer,[57] MacIntosh,[58] and others.

Boeke [59] stated that from a cytologic point of view the nerve endings are in many aspects one of the most interesting parts of the nervous system, and formulated several questions and statements (page 243, *l.c.*) which are a challenge to the anatomists in the study of the neuro-

muscular apparatus. The morphologic evidence on the changes in the denervated neuromuscular apparatus presented in this paper supports the prophetic challenge of Boeke and furthermore substantiates the theory of chemical transmission of nerve impulses advanced by Loewi, Dale, and others. The motor end-plates in living muscle appear to be biologic jet-pumps that discharge either normal or abnormal neurogenic substances into the myoplasm of the muscle fibers.

Summary

1. The right gastrocnemius muscles of 100 white rats (*Mus norvegicus*) were denervated by sectioning the vagus nerve at the hip. The left legs remained intact and were used as controls. The successful demonstration of the nature of the union of nerve endings and voluntary muscle depended upon the use of the gold-and-teasing method of whole muscle fibers and nerves, checked against results obtained by other histologic technics. Gold had an affinity for acetylcholine and certain degeneration products of the axon and myelin.

2. The dark muscle fibers in normal voluntary muscle, because of their strong affinity for gold, are designated as hyperchrysophilous muscle fibers. They are associated with the periodic discharge of acetylcholine from the motor end-plates and with the diffusion of this chemical transmitter in the myoplasm at the end of relaxation or the onset of contraction.

3. The light voluntary muscle fibers have a weak affinity for gold and are designated as hypochrysophilous muscle fibers. They are associated with the periodic hydrolysis of acetylcholine by cholinesterase during the period of full, active contraction.

4. After denervation there is a progressive loss of the dark hyperchrysophilous muscle fibers and motor end-plates, which involved 60 per cent of them 72 hours after denervation. This coincides with the beginning of fibrillation and with the progressive disappearance of acetylcholine. At about the 10th to 15th day after denervation dark muscle fibers are totally absent. The atrophic muscle fibers become achrysophilous. The rates of these pathologic changes vary among animals of the same species as well as among different species.

5. There is a progressive and centrifugal transport of degenerative materials from the distal segment of the sectioned nerve to the denervated muscle. This produces large pleomorphic neurosomes in the denervated myoplasm. This phenomenon is accelerated by the use of d-tubocurarine chloride, particularly when followed by electrical stimulation of the distal stump of the sciatic nerve. There is a substantial

transfer from nerve to muscle. Giant muscle fibers are produced occasionally, composed largely of neurogenic material. The giant fibers of primary muscular atrophy and dystrophy need reinvestigation.

6. The bloated subsarcolemmal nuclei of the denervated muscle become surrounded by an increased amount of granular material coincident with the persistent activity of cholinesterase, as found by Nachmansohn and John,[7] 3 days after nerve section when conductivity had disappeared in the axon.

7. The hyperchrysophilous neurosomes may be defined as ephemeral pleomorphic bodies discharged into the myoplasm of striped muscle from superpermeable terminal axons of motor end-plates. These neurosomes are composed of very fine granules, droplets, vacuoles, and elongated streamers that vary in size and staining capacity with gold, silver, and lipoidal stains.

8. Motor end-plates in living muscle appear to be biologic jet-pumps that discharge neurogenic substances into the myoplasm of the muscle fibers. Voluntary muscle has a dual composition of neurogenic and myogenic substances.

9. Muscle atrophy appears to depend upon a decrease or total inhibition of the neurogenic discharge into muscle, and hypertrophy upon an increase of this neurogenic discharge. The changes in the blood supply appear to be secondary phenomena.

10. Anatomic evidence of progressive degeneration, as contrasted with simultaneous degeneration, occurs in the distal stump of the sciatic nerve. This process may be accelerated by experimental means with resulting massive discharge of degenerated nervous material into the denervated muscle.

11. The union of nerve and muscle appears to be one of periodic anatomic continuity which results in the confluence and compounding of the neurogenic and myogenic substances. Gold chloride, after initial acidulation of the living muscle, appears to possess a selective affinity for the normal and abnormal neurogenic substances.

12. The experimental anatomic evidence appears to support the claim that there are morphologic as well as chemical transmitters of normal and abnormal nerve impulses demonstrable by selective histologic methods.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

The photomicrographs of Figures 1 to 82 are from teased whole muscle fibers (gastrocnemius muscle) and motor end-plates of the white rat *(Mus norvegicus)*, previously prepared by the gold technic. The photographs were direct contact prints from negatives exposed through the microscope and not subsequently enlarged. They may, therefore, be compared with those of the white rat and the chameleon previously published.[1,2] In the plates, "epa" designates the epilemmal axon; "hya," the hypolemmal axon; "Ns," neurosomes or neurogenic particles discharged from motor end-plate jet-pumps into the myoplasm either as fine, nebulous sprays, large agglutinated clusters, or periodic series of globules; "Kg," extra-axonic Kühne's granules; "gmf," giant muscle fiber; "b.v.," blood vessel; "nuc.," nucleus; "gran.," granules. There has been no retouching of negatives or prints. The time after section of the nerve is designated in each legend.

PLATE 241

FIG. 1. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of a rat killed by ether. There is pleomorphism and variation in size and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow, dark muscle fibers may be the initial phase of a nebulous and periodic diffusion of granular acetylcholine at the onset of contraction. Choline and acetylcholine react with gold chloride *in vitro* to produce either choline aurichloride or acetylcholine aurichloride. The wide, light muscle fibers may represent the phase of contraction after the diffused acetylcholine has been destroyed by the cholinesterase. This normal variation of muscle fiber types may be the morphologic expression of the fractional contraction in a muscle, and the differential diffusion alternating with the destruction of acetylcholine in the different fibers of the same muscle. The motor end-plates in the dark muscle fibers are retracted and surrounded by the granules of Kühne. In the light muscle fibers the motor end-plates are usually expanded and there is a decrease in the amount of the granules of Kühne. × 250.

FIG. 2. Spray of medullated nerve fibers depleted of motor end-plates in the gastrocnemius muscle 72 hours after the degenerative cut of the sciatic nerve. The motor end-plates are replaced by fine granules surrounding light, oval spaces occupied by the nuclei of the sole. The epilemmal axons are increased in diameter, and in some terminals there are swollen cysts. The absence of the dark type of muscle fibers in this microscopic field may possibly be related to the failure of normal transmission of acetylcholine granules or the failure of the normal nebulous diffusion of the granular transmitter substance after discharge from the end-plates into the myoplasm. × 250.

FIG. 3. Spray of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 120 hours after the degenerative cut of the sciatic nerve. The muscle was curarized by the intraperitoneal injection of d-tubocurarine chloride and the distal segment was then stimulated for 30 seconds at the rate of 5 per second with no muscular response. There is depletion of the material in the epilemmal axons and an enormous accumulation of this neurogenic material in the end-plate with periodic discharges into the muscle fiber. The exhaustion from the axonic spray of its specific substance with an affinity for gold and the accumulation of this substance at the end-plate and in the muscle constitute conclusive evidence of the substantial transfer of some substance from nerve to muscle. × 250.

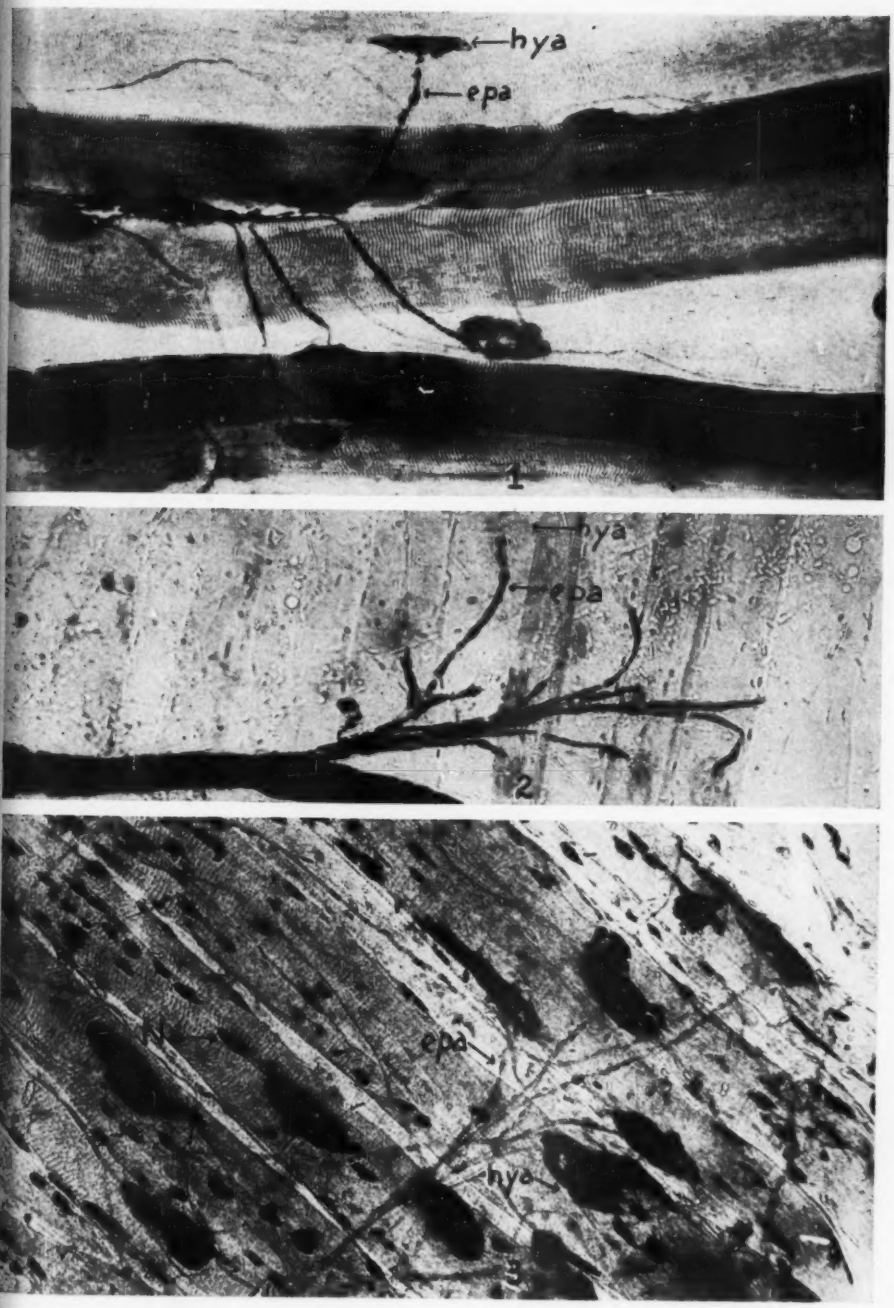
hya
epa
1
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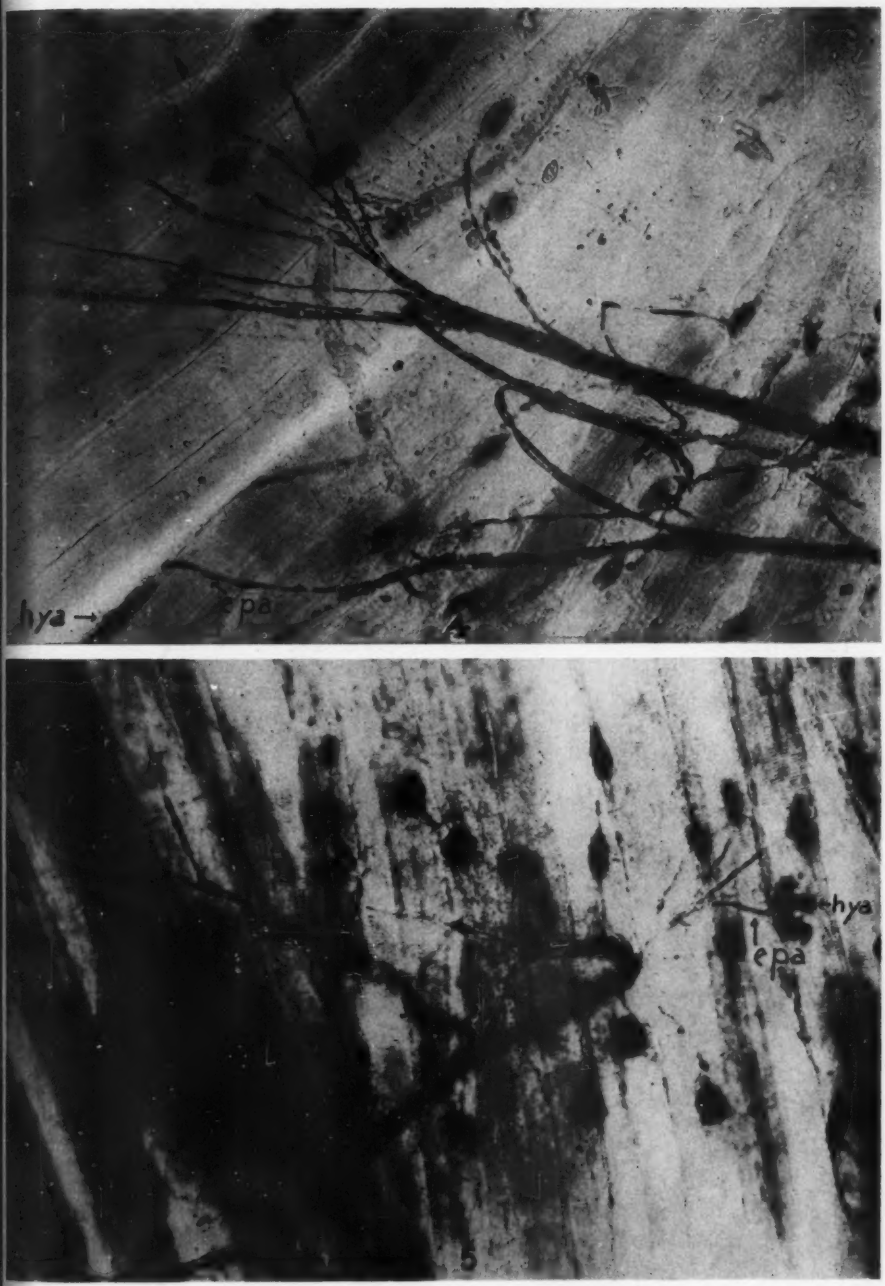
PLATE 242

FIGS. 4 and 5. Sprays of medullated nerve fibers and motor end-plates 24 hours after section of the sciatic nerve. There is a beginning loss of the dark type of muscle fiber. The epilemmal axons are definitely beaded (Fig. 4). In some places the end-plates have a diminished affinity for the gold. In other places in the same muscle all of the end-plates are retracted and have an increased affinity for gold (Fig. 5). Some hypolemmal axons have round or oval cystic enlargements. This is a histologic sign of an abnormal and uniform stimulation of the motor units of nerves and a beginning loss of the fractional contraction of the fibers in the same muscle. In other locations of the same muscle all of the end-plates in the field may be abnormally expanded. The abnormal contraction or expansion of all of the motor end-plates in a field is evidence of uniform overstimulation and overexcitation of the motor units of the muscle and loss of the fractional contraction of the muscle fibers normally controlled by the intact nervous system. × 200.



Carey and Associates Structure of Nerve Impulse Transmitter

PLATE 243

FIGS. 6 and 7. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 48 hours after the degenerative cut. There is a progressive loss of visible structural organization in muscle fibers of the dark type. In many places all of the motor end-plates in a specific field may be either retracted with increased affinity for gold (Fig. 6) or expanded with a decreased affinity for gold (Fig. 7). This is a histologic sign of an abnormal and uniform overstimulation of the motor units and a loss of the fractional contraction of the muscle fibers. There is a dark granular or flaky appearance of the muscle fiber, which is a histologic sign of the beginning loss of the normal nebulous diffusion of the granular transmitter substance discharged from the motor end-plate into the myoplasm of the muscle fiber. × 250.

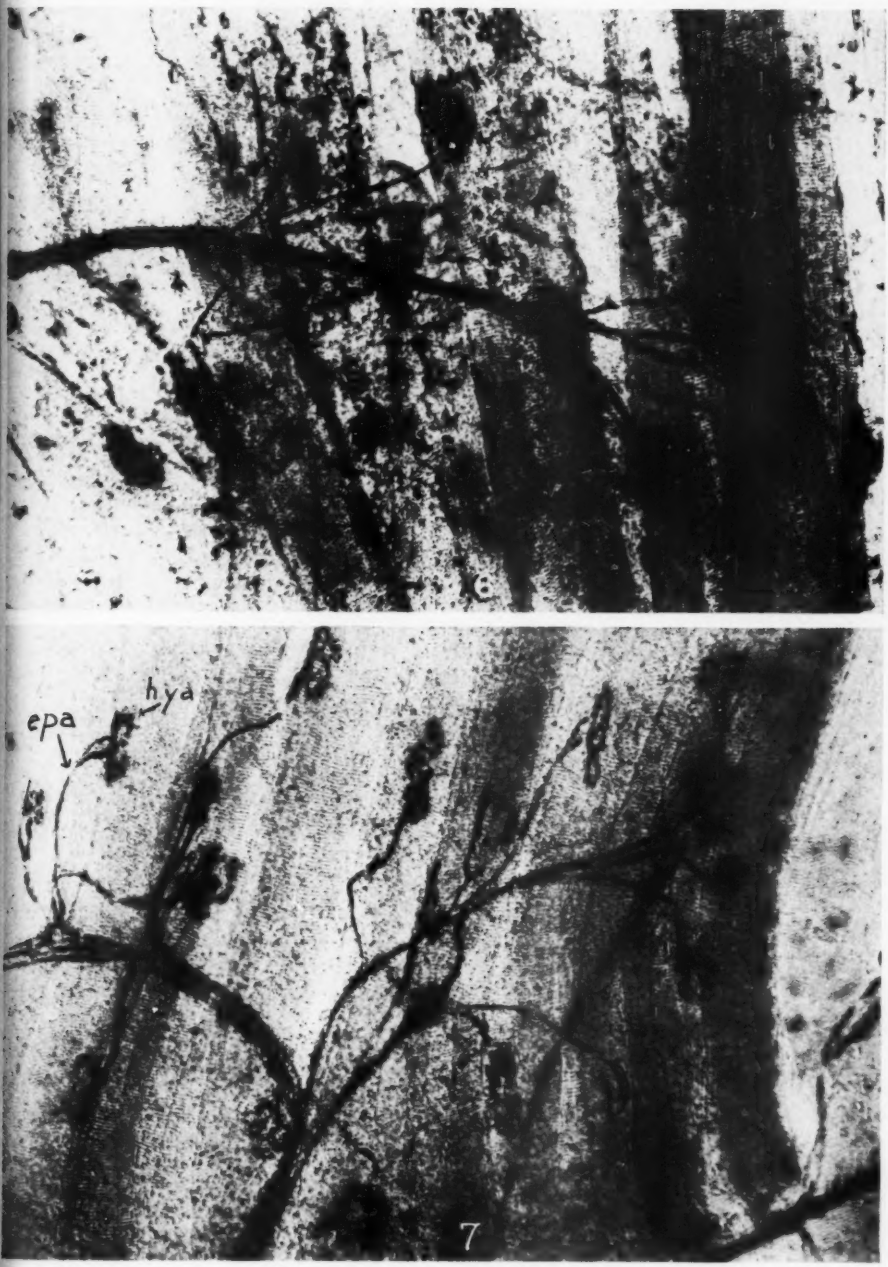

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PLATE 244

FIGS. 8 and 9. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 48 hours after the degenerative cut. There has been an advance in the loss of structure of the muscle fibers. Some of the hypolemmal axons are replaced by fine granules. In some places there is a dark, coarse, granular to flaky appearance of the muscle fibers which is a histologic sign of the beginning loss of the normal periodic diffusion of the fine granular transmitter substance. In some nerve trees the epilemmal axons are fragmented and have a decreased affinity for gold (Fig. 8), while in other places there is an increased capacity to take the gold with little evidence of fragmentation (Fig. 9). Since the technic was standardized by running the normal and abnormal muscles simultaneously through the same fluids for the same periods of time, this is considered a histologic sign of a periodic decrease alternating with an increase in the quantity of material in the epilemmal axons. There is a definite hyperemia of the intramuscular blood vessels and capillaries, demonstrated by the increased diameter of the lumen and the packing of these vessels with red blood cells. In other locations the capillaries are collapsed and devoid of cellular elements. This is comparable to the conditions produced by experimental traumatic and thermal shock and by the local injection of lactic and other acids into the muscle. × 250.

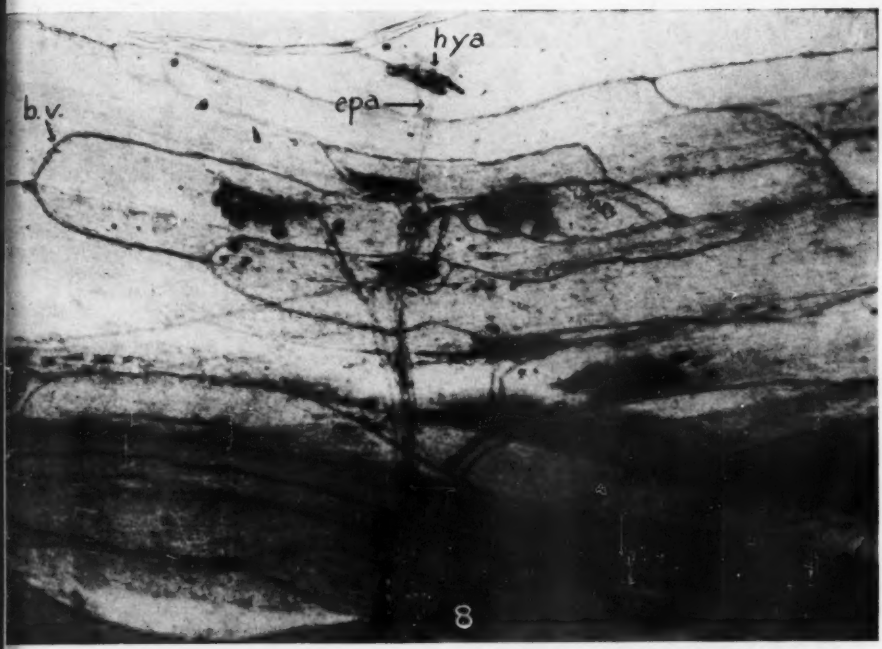
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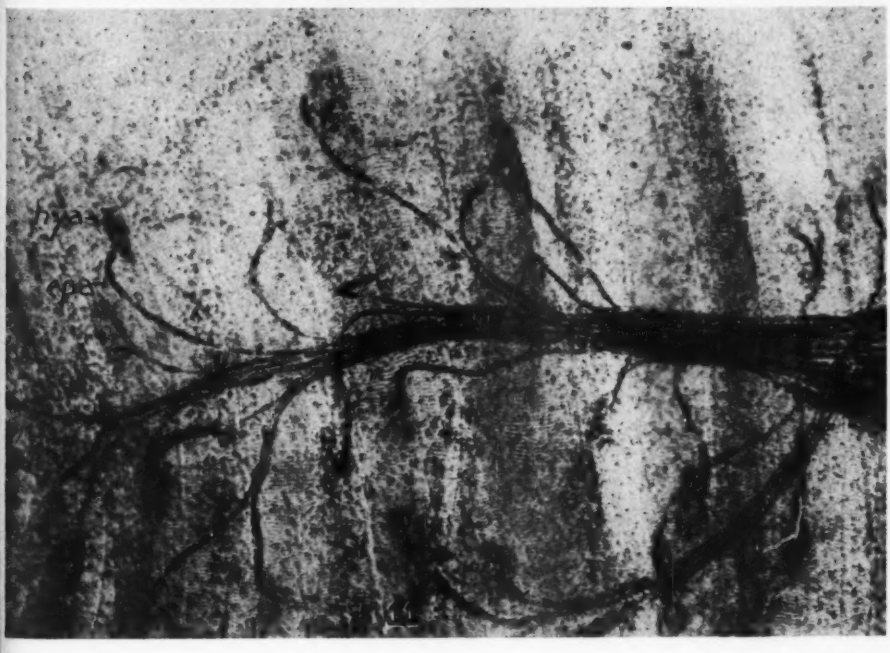
PLATE 245

FIG. 10. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of a rat killed by ether. There is pleomorphism and variation in the size and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow dark muscle fiber associated with a dark retracted end-plate may represent the evanescent phase of the nebulous diffusion of the fine granular acetylcholine at the onset of the contraction of the muscle fiber. The wide light muscle fibers frequently associated with expanded end-plates with a decreased affinity for gold may represent the phase of active contraction of the muscle fiber after the diffused acetylcholine has been hydrolyzed by cholinesterase. This alternation of dark and light auriphilic fiber types may be the morphologic expression of fractional contraction in a normal muscle, and of normal periodic diffusion, alternating with destruction, of the fine granules of acetylcholine in different fibers of the same muscle. The differential capacity to take gold may be a morphologic measure of the quantity of neurogenic substances diffused into the myoplasm of the muscle fiber. The normal and abnormal muscles were concurrently subjected to the same standardized technic. × 250.

FIG. 11. Spray of medullated nerve fibers and granular motor end-plates in the gastrocnemius muscle 72 hours after section of the sciatic nerve. The motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. The epilemmal axons in this field have a decreased affinity for gold in comparison to the relatively normal axons (Fig. 10). There is a dark, coarse, granular or flaky appearance of the muscle fibers, which is a histologic sign of beginning loss of normal diffusion of the fine granules of the transmitter substance discharged from the motor end-plate into the myoplasm of the muscle fiber. There is likewise a definite loss of structure in the dark type of muscle fiber. This may well represent the loss of the normal initial phase of periodic diffusion of the normal neurogenic transmitter substance associated with the production of dark muscle fibers. These dark muscle fibers progressively disappear after the degenerative cut of the nerve, and their disappearance parallels the loss of transmission of acetylcholine from the motor nerve endings into muscle. × 250.



Carey and Associates Structure of Nerve Impulse Transmitter

PLATE 246

FIG. 12. Spray of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 5 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. There is a loss of structure in the dark type of muscle fibers. The fibers are becoming narrower than normal and the sarcolemmal nuclei are more evident in some places than in normal muscle with the gold technic. In some places there is definite fragmentation of the epilemmal axons and in others a depletion of the axonic material. × 250.

FIG. 13. Spray of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 7 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules. The nuclei of the sole plate are beginning to take the gold stain in certain places. There is a loss of structure of muscle fibers of the dark type. The fibers are becoming narrower than normal and the visibility of the sarcolemmal nuclei in some places is increased beyond the normal with gold. In certain places there is definite fragmentation of the epilemmal axons, and in others a depletion of the axonic material. The axonic material in the nerve trunk is fragmented into rounded droplets, elongated bodies with rounded ends, and fusiform structures. In following a nerve trunk throughout its course, first without and then within a muscle, this fragmented axonic material appears to travel in a periodic manner in a centrifugal direction. This material is then discharged from the distal nerve terminal into the muscle. × 250.

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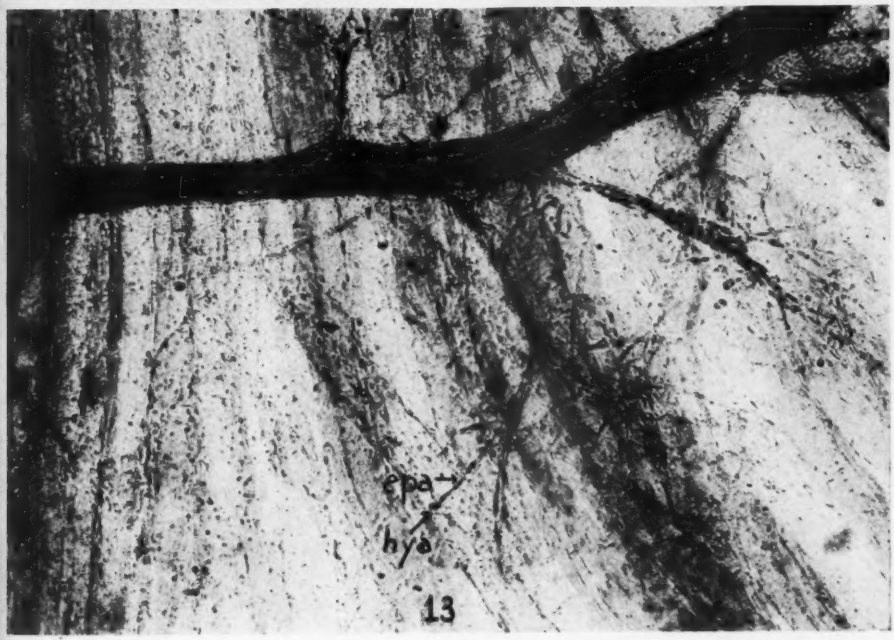
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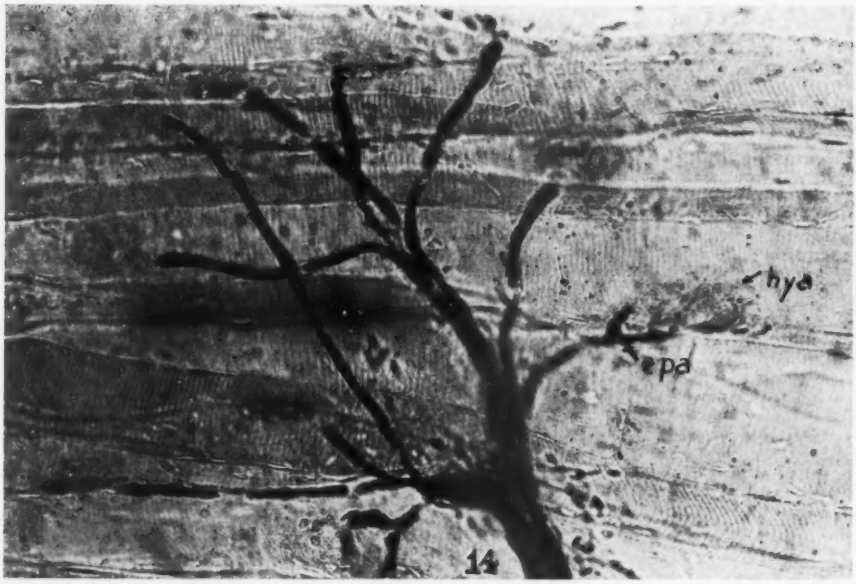
PLATE 247

FIGS. 14 and 15. Sprays of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 9 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. There is a definite loss of the dark type of muscle fiber. In these sprays the epilemmal axons are loaded with material that has a strong affinity for gold. There is very little evidence of fragmentation of the epilemmal axons. After the degenerative cut of the nerve the fragmentation of the epilemmal axon appears and disappears with the progressive and intermittent advance in a peripheral direction of the degenerated axonic material and its discharge centrifugally in the region of the degenerated motor end-plates. There is no evidence of hypolemmal axons. These have been completely replaced by rounded and oval islands of granules. Some of the epilemmal axons have dichotomous divisions. In other places the epilemmal axon is single and terminates in a rounded or frayed end which gradually disappears by confluence with the myoplasm of the muscle fiber. Figure 14, × 400; Figure 15, × 850.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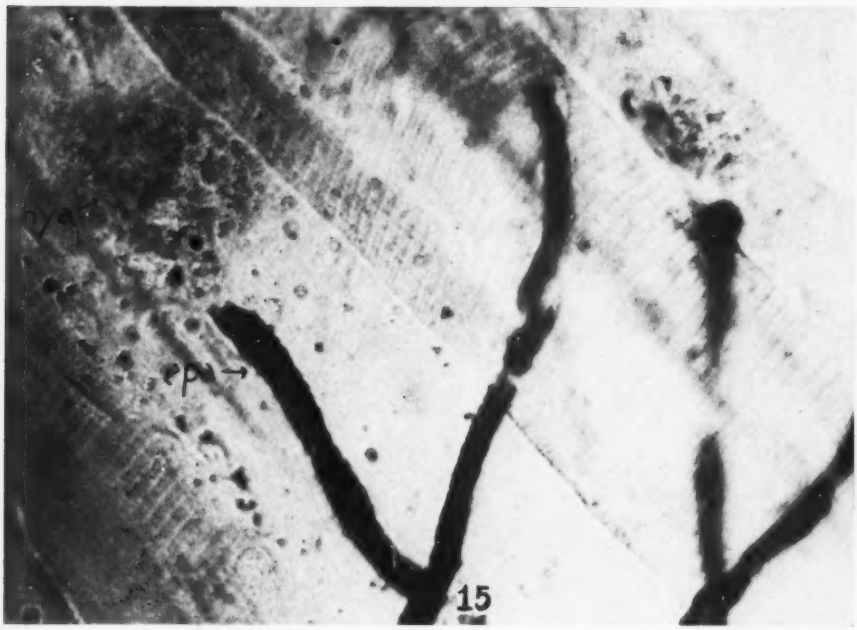
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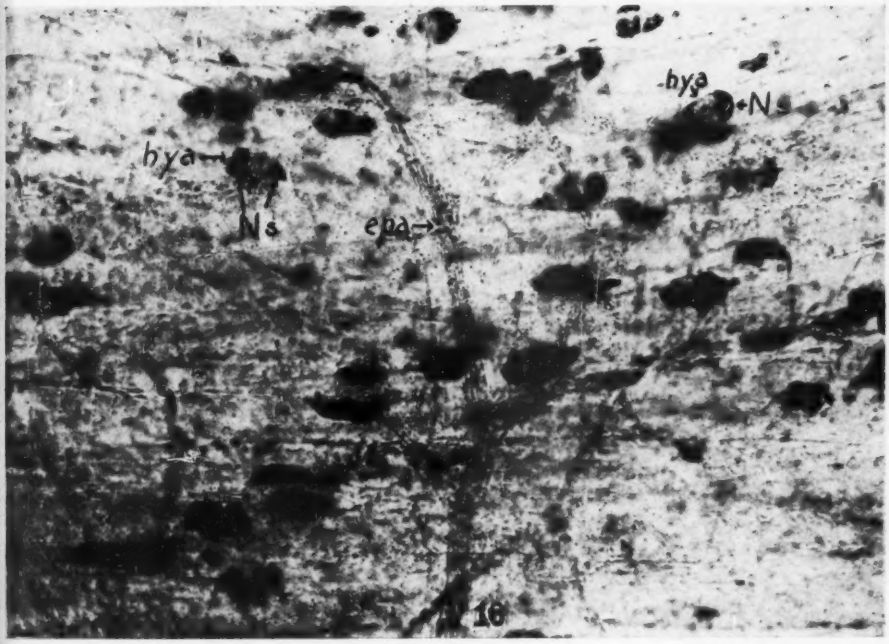
PLATE 248

FIGS. 16 and 17. Sprays of medullated nerve fibers undergoing depletion of their axonic material in a centrifugal direction in a gastrocnemius muscle 10 days (Fig. 16) and 12 days (Fig. 17) respectively after the degenerative cut of the sciatic nerve. The discharged axonic material that has a strong affinity for gold is accumulating in pleomorphic masses within and around the region of the degenerated motor end-plates. The faint, ghost-like outline of the epilemmal axons depleted of substances that have a strong affinity for gold is in striking contrast to that of the accumulated material, with the capacity to take the gold, discharged in a centrifugal direction into the region of the degenerated motor end-plates. × 250.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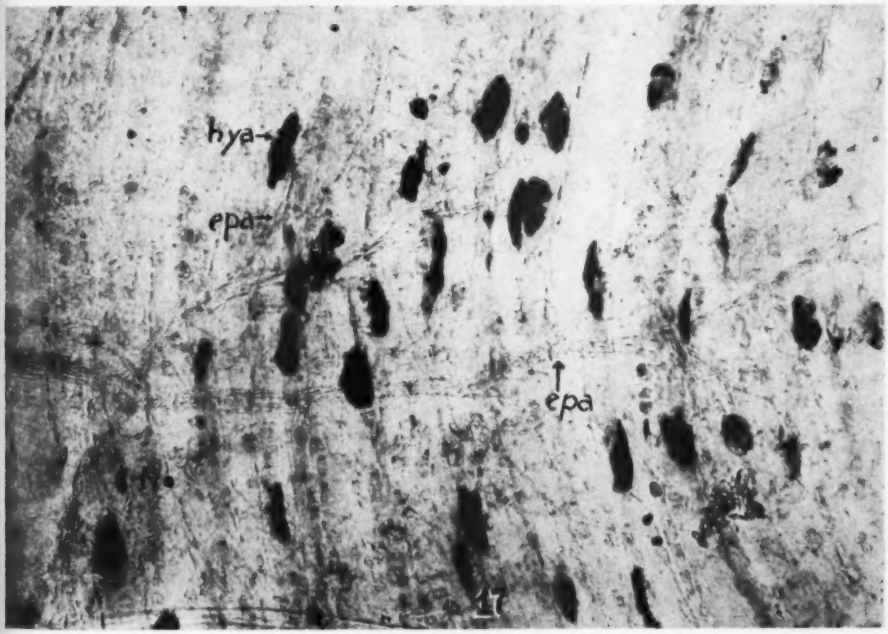
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PLATE 249

FIGS. 18 and 19. Sprays of medullated nerve fibers depleted of their axonic auriphilic material which is discharged in a centrifugal direction into the gastrocnemius muscle 14 days (Fig. 18) and 20 days (Fig. 19) respectively after the degenerative cut of the sciatic nerve. The discharged axonic material with a strong capacity to combine with gold is accumulated in pleomorphic masses within and around the region of the degenerated motor end-plates. Some of these pathologic masses or neurosomes are found scattered in the myoplasm of the muscle fiber. The faint, ghost-like outline of the epilemmal axons depleted of their substance that has an affinity for gold is again in striking contrast to the accumulated gold-impregnated material discharged in a centrifugal direction. The muscle fibers 20 days after the degenerative cut have lost the appearance of the normally functioning dark type, and they are considerably narrower in comparison with the normal. The anatomic evidence is incontrovertible that the histologic dark type of muscle fiber is related to a normally functioning nerve supply. This functional dark type of muscle fiber appears to be produced by the normal diffusion into the myoplasm of the fine granules of acetylcholine discharged from the motor end-plate. (For comparison with Figs. 1, 10, and 22). × 250.

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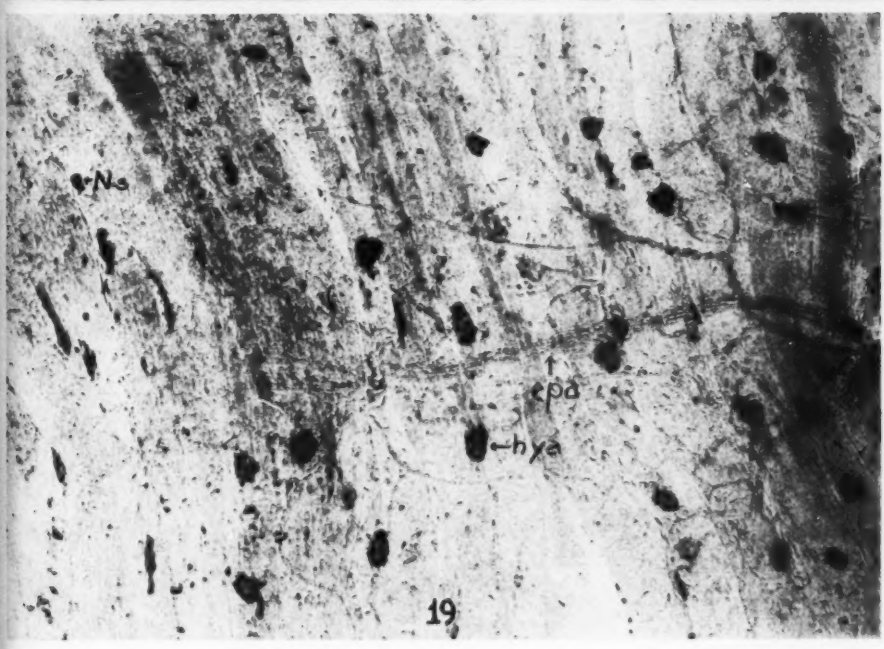
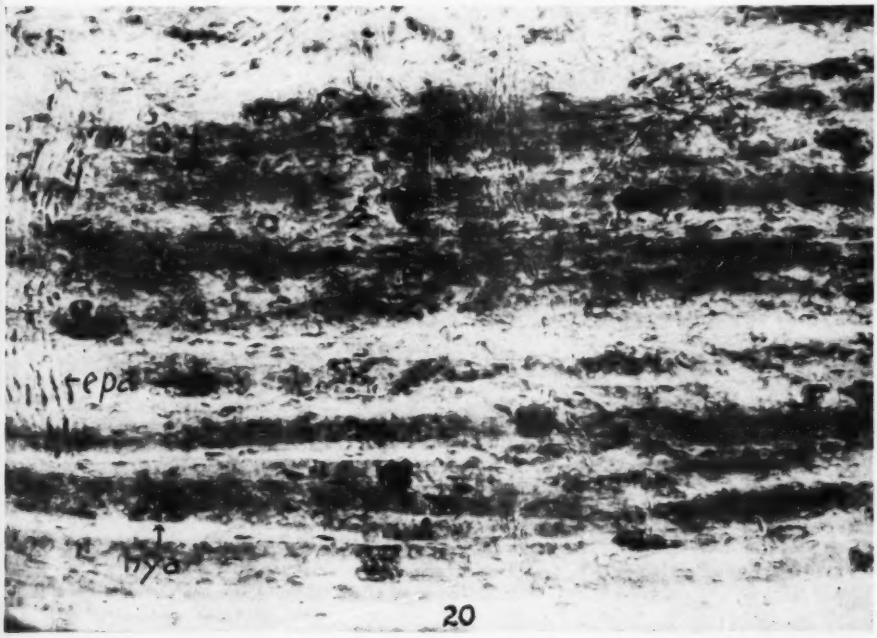
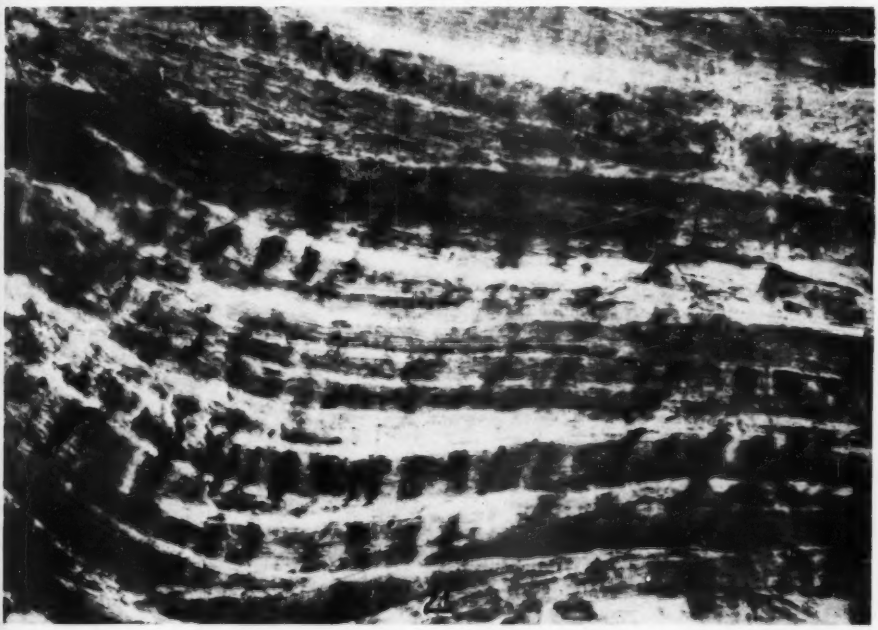


Carey and Associates Structure of Nerve Impulse Transmitter

PLATE 250

FIGS. 20 and 21. Sprays of medullated nerve fibers depleted of their axonic material in the gastrocnemius muscle 30 days after the degenerative cut of the sciatic nerve. The axonic material, which was discharged in a centrifugal direction, is seen only in relation to a few of the degenerated end-plates. The locations of the degenerated end-plates are indicated principally by clusters of sole-plate nuclei which are surrounded by the granular material secreted by these nuclei. This granular material has an affinity for gold slightly less than that of the material discharged by the hypolemmal axons. The clusters of nuclei are detected as clear, rounded, or oval spaces surrounded by dark granules (Fig. 20). In some places, in the same muscle, the myoplasm is aggregated into broad or narrow, dark, transverse bands alternating with light ones (Fig. 21). In the dark bands the cross striations are either exceedingly fine or they are completely absent because of the opacity of the dense aggregation of the myoplasm. The light bands are occupied by cross striations which are farther apart than those in the dark bands. This dark and light transverse banding of the narrow muscle fibers is produced by the slow, irregular fibrillation of the muscle fibers. This incoordinate, ceaseless, slow, vermiform fibrillation of the denervated muscle fibers begins about 72 hours after the degenerative cut of the sciatic nerve, and is correlated with loss of transmission of acetylcholine, which, normally, chemically tunes the muscle fiber to the higher pitch or frequency of fast muscle contractions and maintains its capacity to respond adequately to high-frequency stimulation. This chemical tuning process is roughly analogous to the mechanical tuning of the strings of a piano or violin for normal response at a higher frequency than is possible in strings sagging between their supports. × 250.





Carey and Associates Structure of Nerve Impulse Transmitter

PLATE 251

FIG. 22. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of the rat killed by ether. There is definite pleomorphism with variation in the size, shape, and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow, dark muscle fibers may represent the initial phase of the fine, nebulous diffusion of granular acetylcholine at the beginning of contraction of certain muscle fibers. The wide, light muscle fibers may represent the phase of active contraction of some muscle fibers after the diffused acetylcholine has been destroyed by hydrolysis through the action of the cholinesterase. This variation and alteration of fiber types in the same muscle fiber may be the morphologic expression of the fractional contraction of the fibers in a muscle and the active periodic diffusion, alternating with the destruction by hydrolysis, of acetylcholine in the different muscle fibers of the same muscle related to a normal innervation. The same muscle fiber at different periods of time may be either dark or light by gold impregnation, dependent upon the phase of active diffusion or hydrolytic destruction respectively of acetylcholine granules. The dark and light muscle fibers, according to this concept, are morphologic variations that parallel the different periods of functional activity. The dark muscle fiber loses that character when the normal secretion of acetylcholine is abolished by denervation. × 150.

FIG. 23. Spray of medullated nerve fibers depleted of their axonic and degenerated myelin materials in the gastrocnemius muscle 10 days after the degenerative cut of the sciatic nerve. There is a definite loss of structure of the different types of muscle fibers. The dark type of muscle fiber has been eliminated. The epilemmal axons are definitely depleted of the fragmented axonic and myelinic materials. This depletion of the epilemmal axons of their gold-impregnated materials is due to the centrifugal discharge of the degenerated axonic substance into and around the degenerated motor end-plates. There is, likewise, a discharge of neurosomes throughout the muscle fibers. This morphologic change was produced by the intraperitoneal injection of d-tubocurarine chloride followed by electrical stimulation of the degenerating peripheral stump of the cut sciatic nerve. The demonstration of the pathologic structure of the normal or abnormal transmitter substance may be demonstrated best experimentally by accelerating the discharge of the axonic material into the muscle and by preventing or delaying its normal diffusion into the myoplasm of the muscle fiber. × 150.

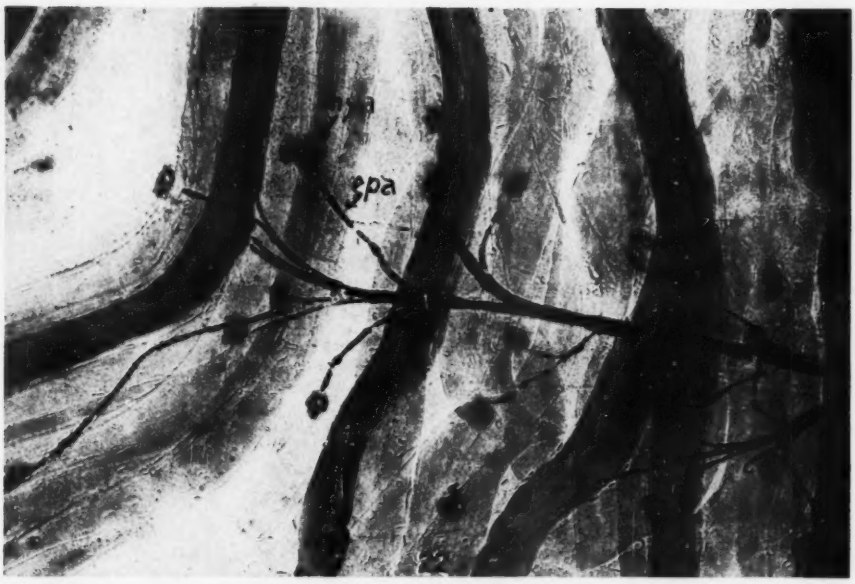

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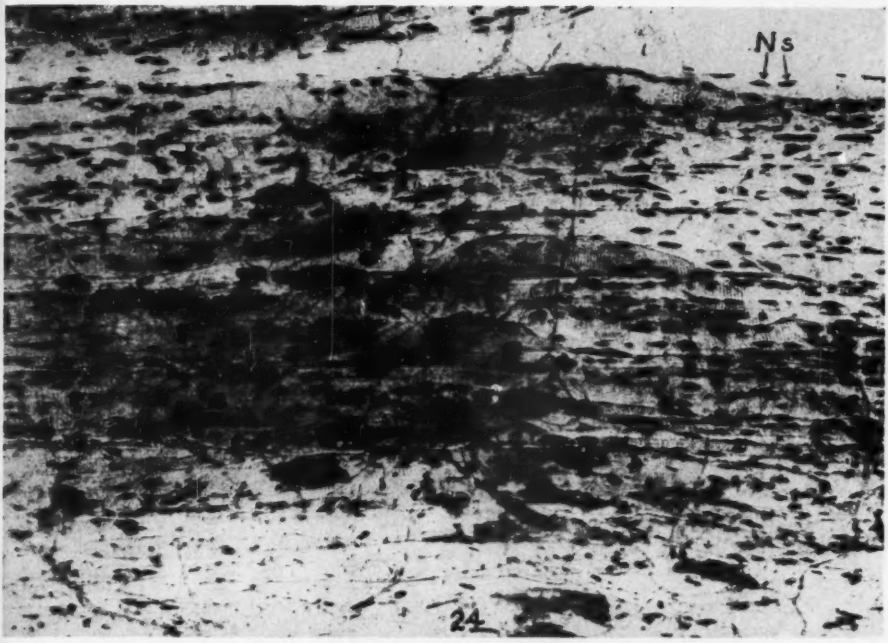
PLATE 252

FIGS. 24 and 25. Sprays of medullated nerve fibers depleted of their axonic and myelin material with the degenerated motor end-plates surrounded by augmented material with a strong affinity for gold, from the gastrocnemius muscles of rats, 12 (Fig. 24) and 14 (Fig. 25) days, respectively, after section of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. There is practically complete depletion of the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has been projected in a very profuse manner throughout the muscle fibers. There is a definite loss of the dark type of muscle fiber as a result of denervation (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the discharge and the lack of normal diffusion of this abnormal transmitter substance into the myoplasm of the muscle fiber. The irregular discharge of the nondiffusible nervous substances into the muscle apparently prevents the appearance of the functional dark type of muscle fiber, which appears to be the product of the discharge and uniform nebulous diffusion in a periodic manner of fine granules from a normal innervation. These fine neurogenic granules may be interstitial granules of Kölliker found in normal muscle. The large neurosomes may represent not only an agglutination of the granules of Kölliker, but also the discharge of abnormal substances into the muscle. × 150.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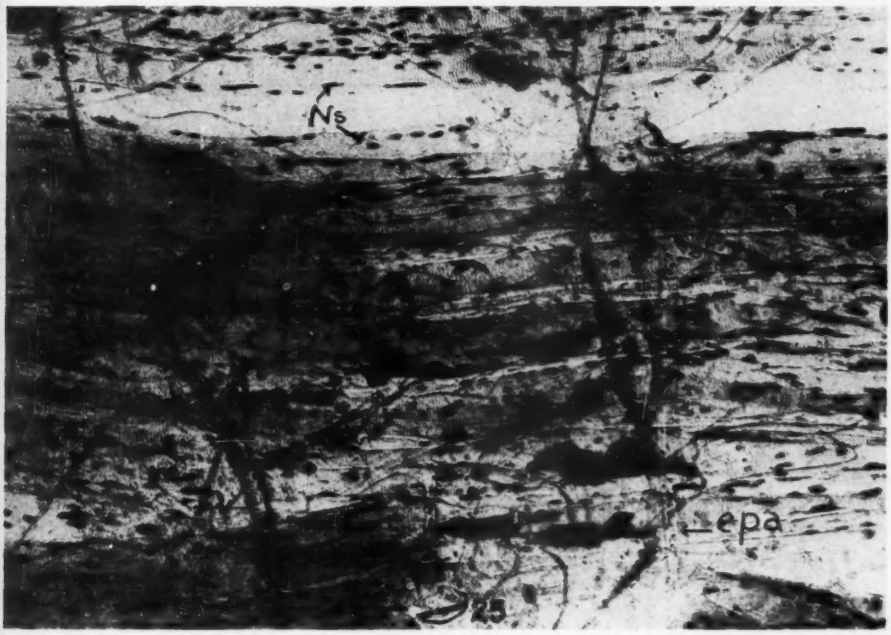
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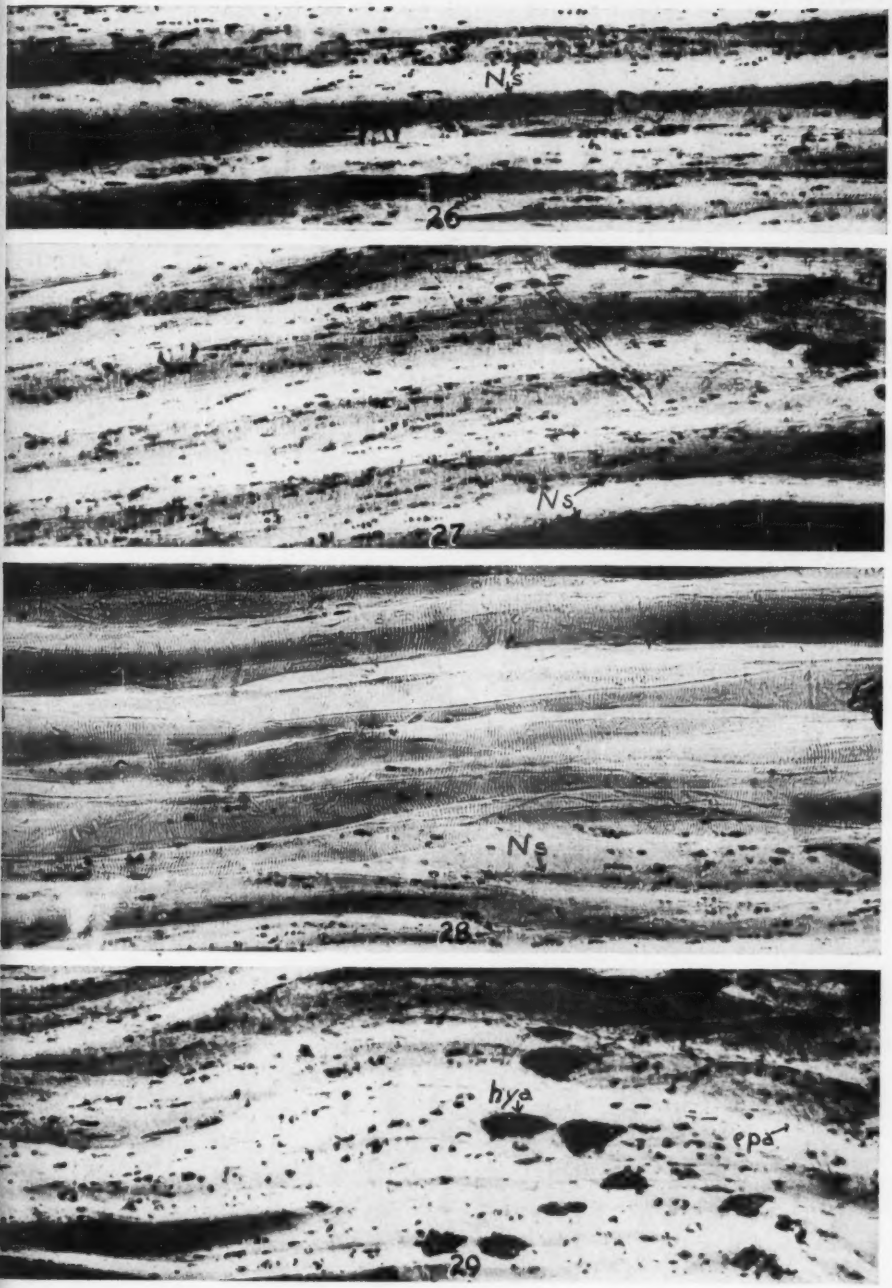
PLATE 253

FIGS. 26 to 29. Sprays of medullated nerve fibers depleted of their axonic and myelin materials, and the degenerated motor end-plates surrounded by an augmented amount of material with a strong affinity for gold in the gastrocnemius muscle of the rat 15 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for a duration of 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has likewise occurred as multiple series of small, round droplets with a strong affinity for gold. Two to ten little droplets that have a strong affinity for gold are found in each series. The beginning and the end of each series is composed of droplets smaller than those at the middle of the series. Some muscle fibers do not contain these droplets of neurogenic material. This pleomórphism of the material discharged by the end-plate is related to the different phases in the abnormal secretion from the motor nerve terminal into the myoplasm of the muscle fibers. × 150.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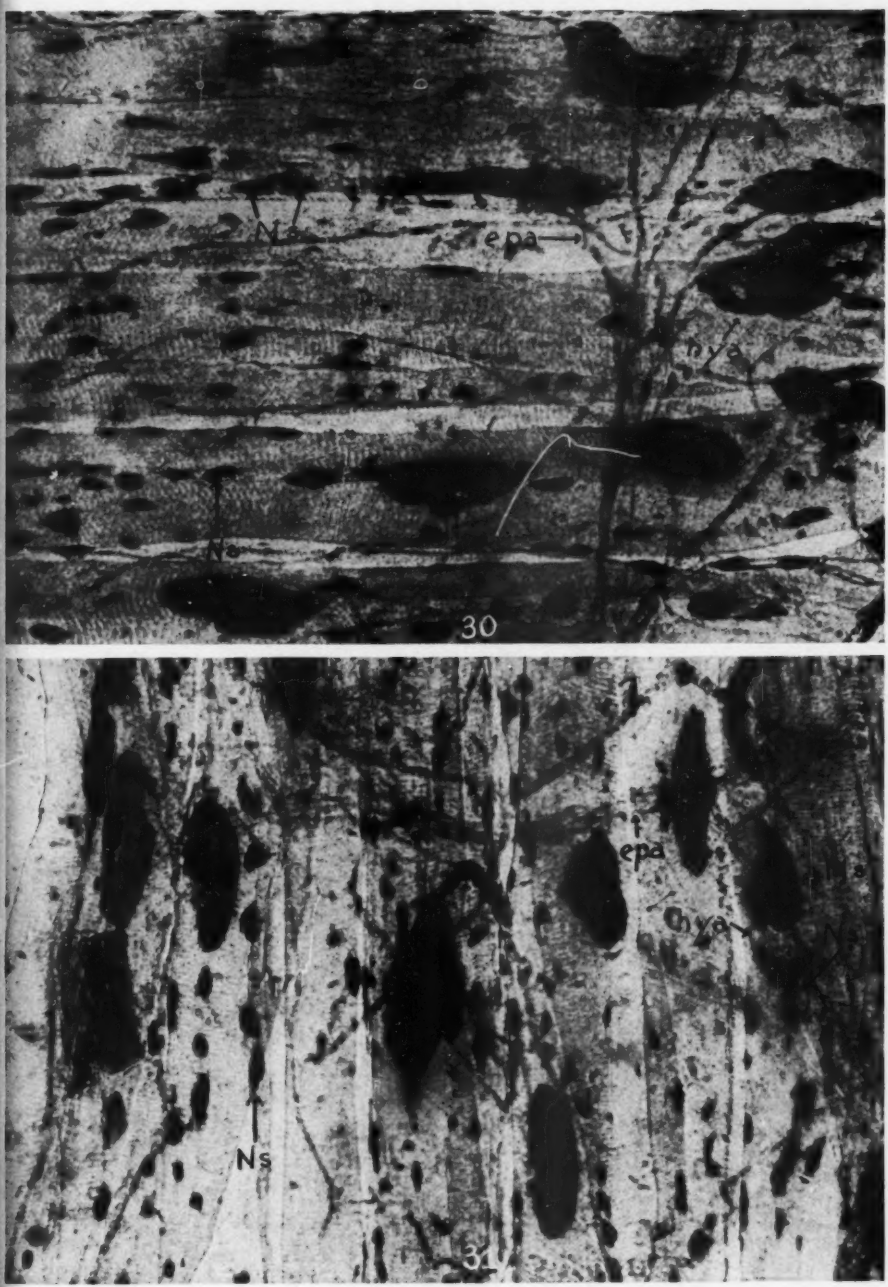
29

PLATE 254

FIGS. 30 and 31. Sprays of medullated nerve fibers depleted of their degenerated axonic and myelin materials, and degenerated motor end-plates surrounded by an augmented amount of substances with a strong affinity for gold in the gastrocnemius muscles of rats, 5 (Fig. 30) and 10 (Fig. 31) days, respectively, after the degenerative cut of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for a duration of 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has, likewise, occurred in a very profuse manner through the myoplasm. There is a definite loss of the functional dark type of muscle fiber (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the abnormal discharge and the lack of the normal nebulous and periodic diffusion of the fine, granular transmitter substance into the myoplasm. The profuse discharge of nondiffusible and abnormal nervous particles into the muscle apparently prevents the appearance of the functional dark type. Three experimental factors are working concurrently to produce the large amount of neurogenic material in muscle: the curare-like effect on muscle of nerve sectioning, the active effect of relatively pure curare upon the myoplasm, and the accelerated discharge of relatively nondiffusible or delayed-diffusible degenerated axonic and myelin materials produced by the electric stimulation of the distal stump of the cut sciatic nerve. With the discharge of great masses of the abnormal axonic material into the muscle there is a concomitant disappearance of this material in both the epilemmal and hypolemmal axons of the motor end-plate. × 350.


epa
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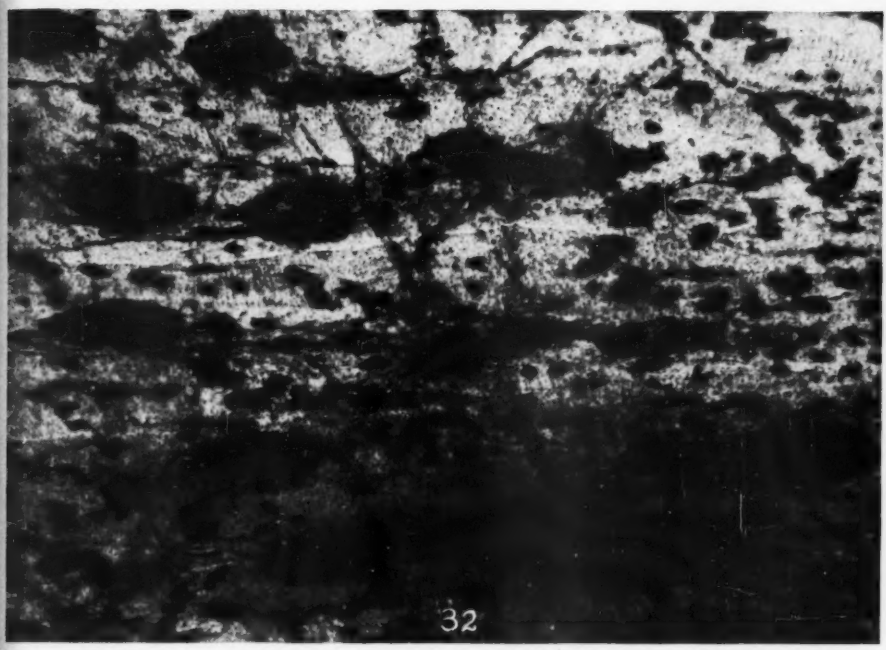
epa
Ns
31

PLATE 255

FIGS. 32 and 33. Sprays of medullated nerve fibers depleted of their degenerated axonic and myelin materials, and degenerated motor end-plates surrounded by an augmented amount of substances with a strong affinity for gold in the gastrocnemius muscles of rats, 15 (Fig. 32) and 20 (Fig. 33) days, respectively, after degenerative section of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has, likewise, occurred in a very profuse manner throughout the muscle fibers. There is a definite loss of the dark type of muscle fiber (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The demonstration of the pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the discharge and the lack of normal diffusion of this abnormal transmitter substance into the myoplasm of the muscle fiber. The irregular discharge of nondiffusible nervous substances into the muscle apparently prevents the appearance of the functional dark type of muscle fiber. There is a definite elongated and fusiform structure assumed by the gold-impregnated material that accumulates around the degenerated motor end-plates about the 20th day after the degenerative cut. This is a mechanical resultant of the atrophy and decrease in diameter of the denervated gastrocnemius muscle fibers. This atrophy of the muscle fibers mechanically molds the liquid or semiliquid neurogenic materials discharged, at the terminals of the degenerated motor nerve, into the muscle fibers. The demonstration of the pathologic structure of the normal or abnormal transmitter substance may be accomplished best, experimentally, by accelerating the discharge of the axonic material into the muscle and by preventing or delaying its normal diffusion into the myoplasm. × 350.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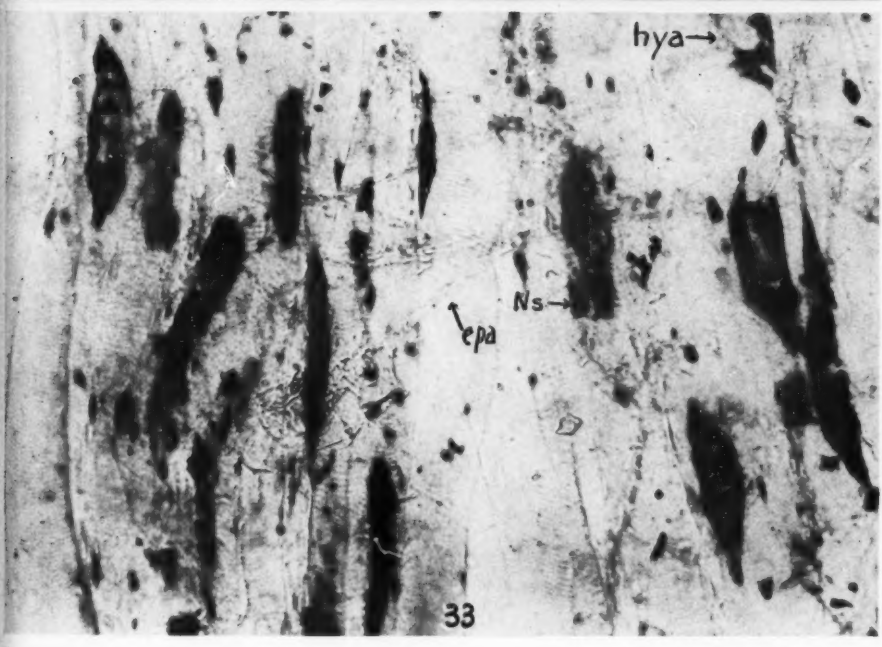
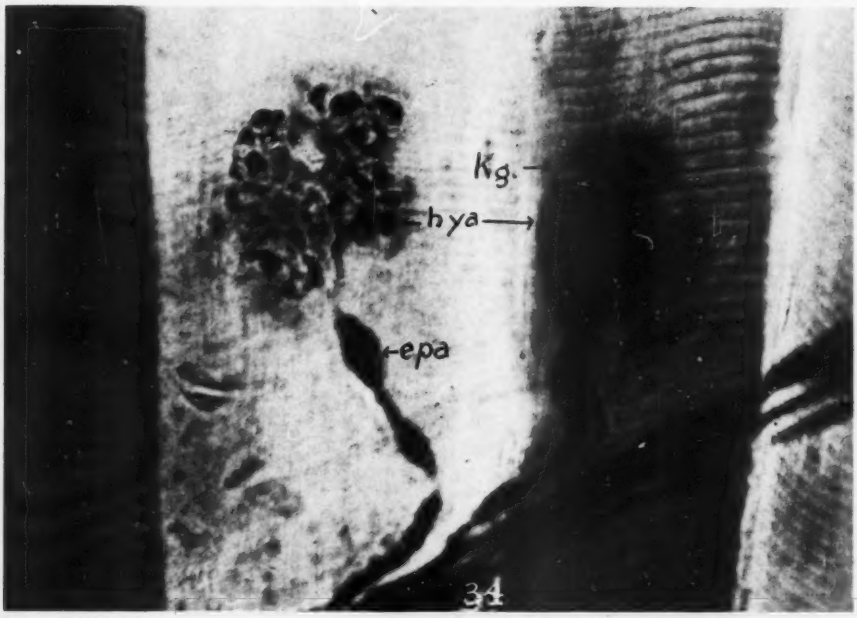

hya→
Ns→
epa
33

PLATE 256

FIGS. 34 and 35. Sprays of medullated nerve fibers and terminal motor end-plates from the relatively normal gastrocnemius muscle of a rat killed by ether. The dark and light muscle fibers are evident. The dark muscle fibers contain more material that has an affinity for gold than the light muscle fibers. The dark, anisotropic, transverse bands in the dark muscle fibers are usually broader and darker than those in the light fibers. On the other hand, the light, isotropic, transverse striations in the light muscle fibers are usually not only broader than the light spaces in the dark muscle fibers, but there is less material that has an affinity for gold in the light spaces of the light muscle fibers than in corresponding cross striations of dark fibers. There are multiple gradations in the width and capacity to take gold of the cross striations. The motor end-plates in the dark, narrow muscle fibers usually possess coarse fronds or knob-like terminals. These fronds may or may not be surrounded by light halo-like spaces, but they are usually surrounded by more dark, granular material of the sole plate of Kühne than in the sole of the expanded end-plates in light muscle fibers. The granules of the sole plate are derived from two sources: from the granular transformation and permeability of the terminals of the hypolemmal axons, and from the nuclei of the sole plate. This granular material of the sole plate normally diffuses in a periodic manner throughout the myoplasm of the dark fiber. The motor end-plate in the light muscle fiber usually exhibits an ameboid expansion and an attenuation of the hypolemmal axons. There is, likewise, a decreased amount of the diffusible granules of Kühne around the motor end-plates of the light muscle fibers. The dark fiber may well represent the stage at the onset of muscle contraction and the light muscle fiber the phase of active contraction. It is practically impossible to catch a muscle fiber in a state of completely unstable relaxation by any histologic technic used to date. The dark muscle fiber probably represents the state nearest to that of relaxation or at the beginning of the stage of contraction of the myoplasm produced by the periodic diffusion of acetylcholine. The dark muscle fibers in the teased specimens have a strong affinity for gold and are stained deep red, blue, or purple. The light muscle fibers have a decreased affinity for gold or are entirely devoid of this staining property, probably because of the temporary absence of chemicals that have an affinity for gold. There are multiple gradations between the hyperchrysophilous and the achrysophilous fibers. × 850.


Kg.
hya
epa
34

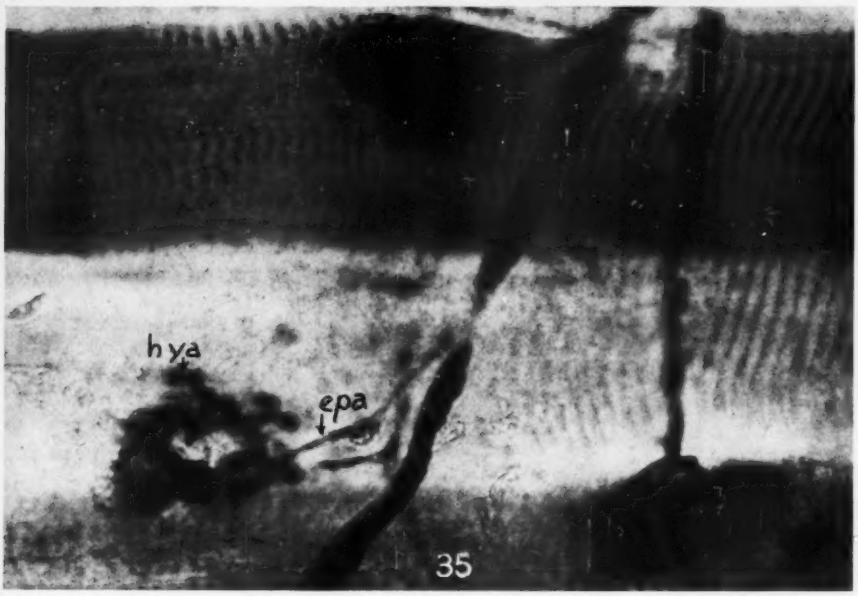
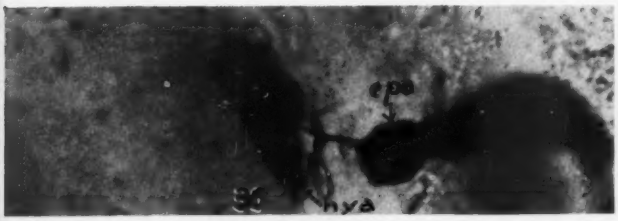

hya
epa
35

PLATE 257

FIGS. 36 to 40. Motor end-plates from the gastrocnemius muscle of a rat 24 hours after the degenerative cut of the sciatic nerve. Some retracted end-plates (Figs. 36 and 37) are associated with very few Kühne's granules but are in continuity with epilemmal axons that are enlarged and form cysts. There appears to be a temporary block of transmission of substance from the epilemmal axon to the hypolemmal ramifications in the nerve ending. Some of the end-plates are relatively normal, but in places there are relatively large, rounded, or oval thickenings, varying in number, size, and shape, which appear on the arborizations of the motor endings. These retention cysts contain materials that have a strong affinity for gold (Figs. 36, 37, and 38). Other end-plates are relatively expanded with ameboid branchings of their terminal arborizations (Figs. 39 and 40). These terminal branchings are more elongated, more narrow, and have a decreased affinity for gold as compared to the relatively retracted endings. The expanded endings, likewise, have a decreased amount of the Kühne granules of the sole plate. The direct transformation of the hypolemmal axons into granules appears to be one origin of the granular sole plate of Kühne. The retracted and expanded nerve endings, therefore, are functional types that alternate in the same muscle fiber at different times and do not represent a specific definitive type of nerve ending that is always constant in its structure. × 850.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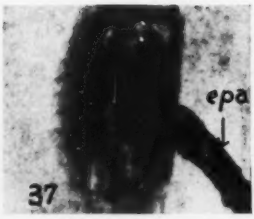
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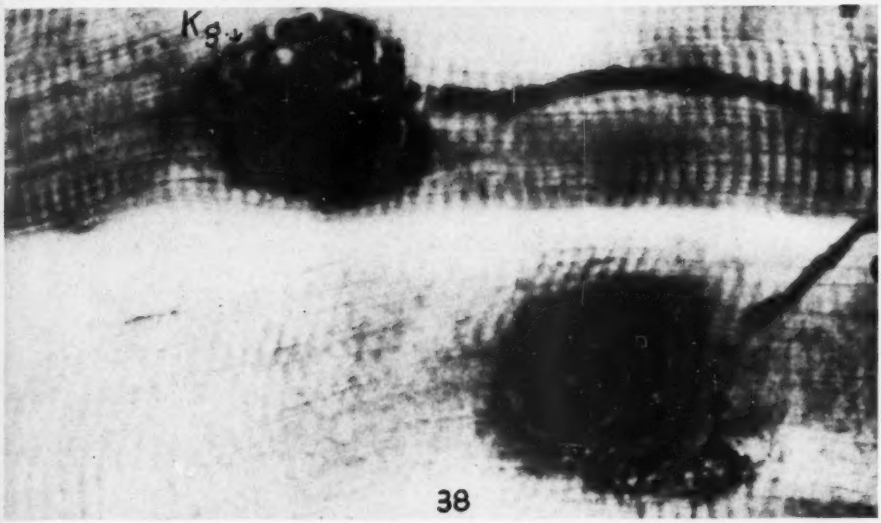

Kg
38

epa

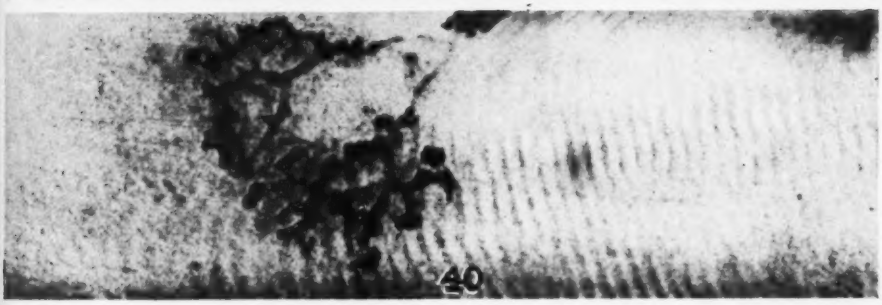
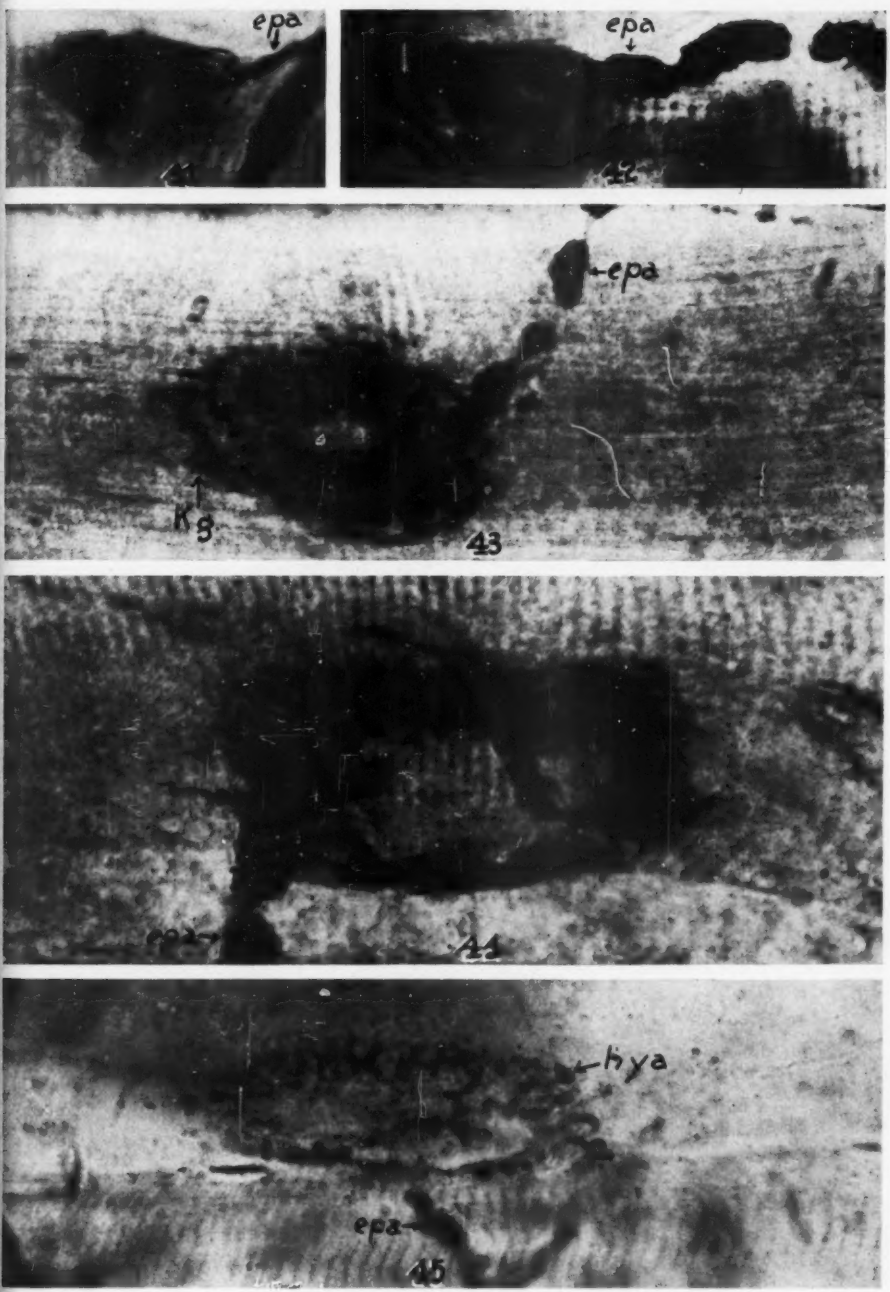

40

PLATE 258

FIGS. 41 to 45. Motor end-plates from the gastrocnemius muscle of a rat 48 hours after the degenerative cut of the sciatic nerve. Some retracted end-plates (Fig. 41) are associated with very few Kühne's granules whereas others (Fig. 42) possess a large amount of surrounding Kühne's granules. There appears to be a cystic enlargement of both the hypolemmal (Fig. 41) and epilemmal (Fig. 42) axons in some places. These cystic enlargements contain materials that have a strong affinity for gold. In other locations of the same muscle (Figs. 43, 44, and 45) there is an expansion of the hypolemmal axons of the motor end-plate. This appears to be accomplished by an ameboid extension of the hypolemmal axons into the myoplasm. In some of these expanded motor endings (Fig. 43) there is found a peripheral disposition of Kühne's granules separated from the axons in some places by a clear, halo-like area, while in others the granules are in direct continuity with the hypolemmal axons. Certain expanded motor end-plates (Fig. 44) have an increased quantity of materials with an affinity for gold. In other locations of the same muscle (Fig. 45) the extremely attenuated and narrowed ramifications of the motor end-plate are undergoing a transformation into granules. They possess a greatly decreased quantity of materials with an affinity for gold. This morphologic change appears to be due to the direct transformation of the motor nerve endings into granules, and the discharge and periodic diffusion of these granules into the myoplasm. Under abnormal overstimulation of a continuous type due to the degenerative cut of the sciatic nerve, this results in a progressive exhaustion of the motor end-plates of their materials that have an affinity for gold. There are 15 single dark cross striations related to the retracted motor end-plate (Fig. 41) and 33 duplex striations related to the expanded end-plate (Fig. 44). It is clearly evident, therefore, that the cross striations do not form constant and fixed relations with the motor end-plate. If they did, they would be mechanically greatly separated during the period of the expansion of the motor end-plate. The cross striations periodically appear and disappear depending upon capillary chemical changes in composition and concentration at the neuromuscular apparatus. This morphologic evidence is not consistent with the theory that there is a constant number of units of sarcomeres in a voluntary muscle fiber or that the *sine qua non* of striated muscle is the continuous existence of a series of so-called sarcomeres composed of ZJQJZ cross bands. These cross striations are produced by the appropriate microcapillary chemical conditions of periodic condensation and normal diffusion of substances, involving the Liesegang phenomenon. They do not depend upon constant and rigid architectural design of a static structure as taught in histology. × 850.


epa
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43
44
hya
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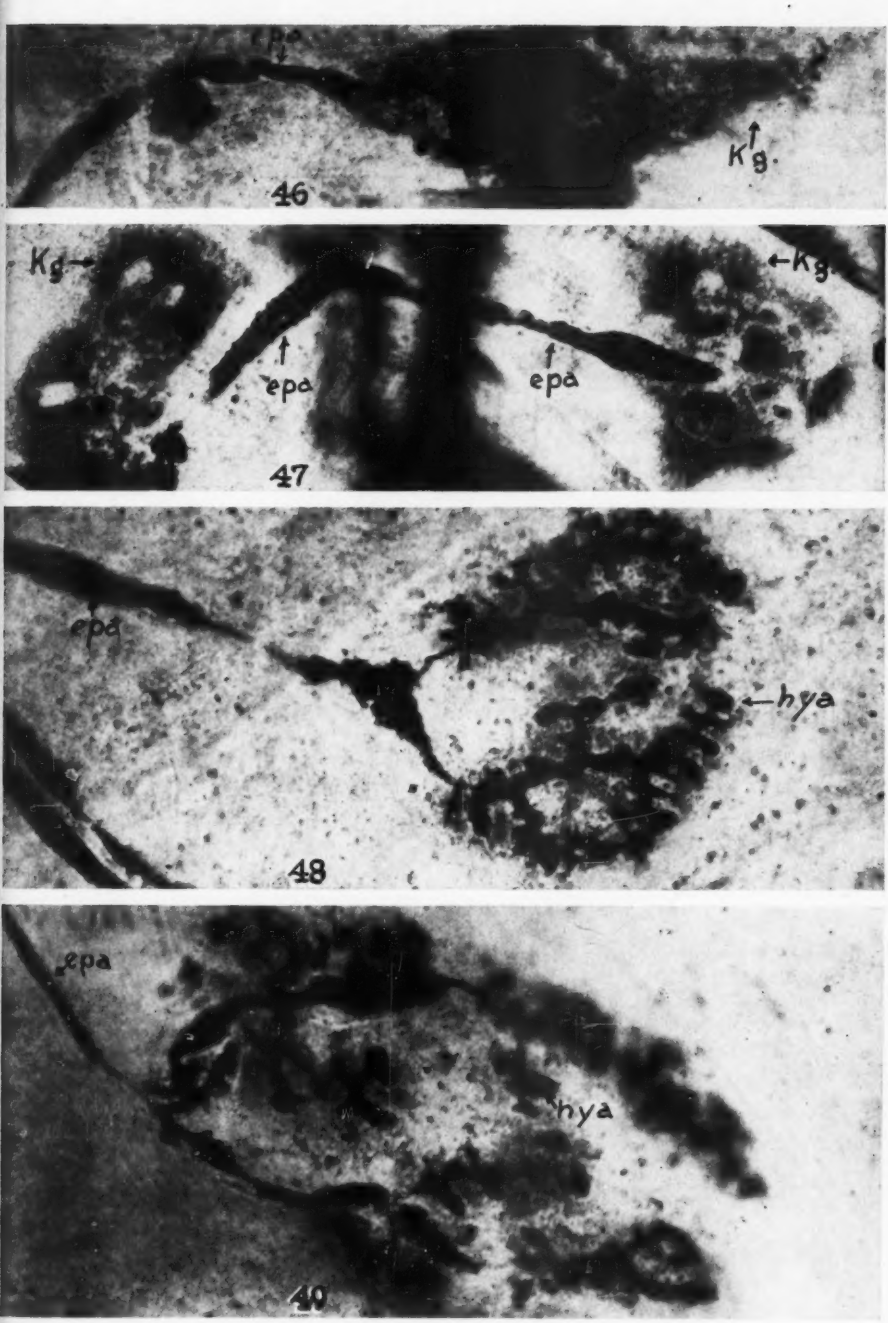
45

PLATE 259

FIGS. 46 to 49. Motor end-plates from the gastrocnemius muscles of rats 24 hours (Figs. 48 and 49) and 48 hours (Figs. 46 and 47), respectively, after the degenerative cut of the sciatic nerves. Some retracted end-plates (Fig. 46) contain a large central island composed of materials that have a strong affinity for gold. Some of the hypolemmal axons have undergone granular transformation and are continuous with the streaming of granules from the sole plate of Kühne out into the myoplasm. Other arborizations of the motor end-plates are enlarged and form cysts containing material with a strong affinity for gold (Fig. 46). In other locations of the same muscle practically all of the hypolemmal axons have undergone a granular transformation. These granules are directly continuous with, and are discharged into, the myoplasm (Fig. 47). Among the granules of the transformed motor end-plates will be found clear, rounded, and oval spaces which contain the nuclei of the sole plate. The epilemmal axons have an irregular contour. In other places there is an enormous enlargement of the motor end-plates by ameboid expansion (Figs. 48 and 49). These expanded terminals have relatively large, rounded, or oval thickenings, which vary in number, size, and shape. These enlargements which appear on the arborizations of the motor endings have a strong affinity for gold. There is no definite granular sole plate but there is evidence, in certain places, of the direct transformation of the end arborizations into granules which diffuse into the myoplasm. In some retracted (Fig. 46) and expanded (Figs. 48 and 49) end-plates the related cross striations of the muscle fibers are replaced by granules. It is clearly evident that more cross striations would be related to the expanded than to the retracted nerve ending if the inconstant cross striations were present. × 850.


epa
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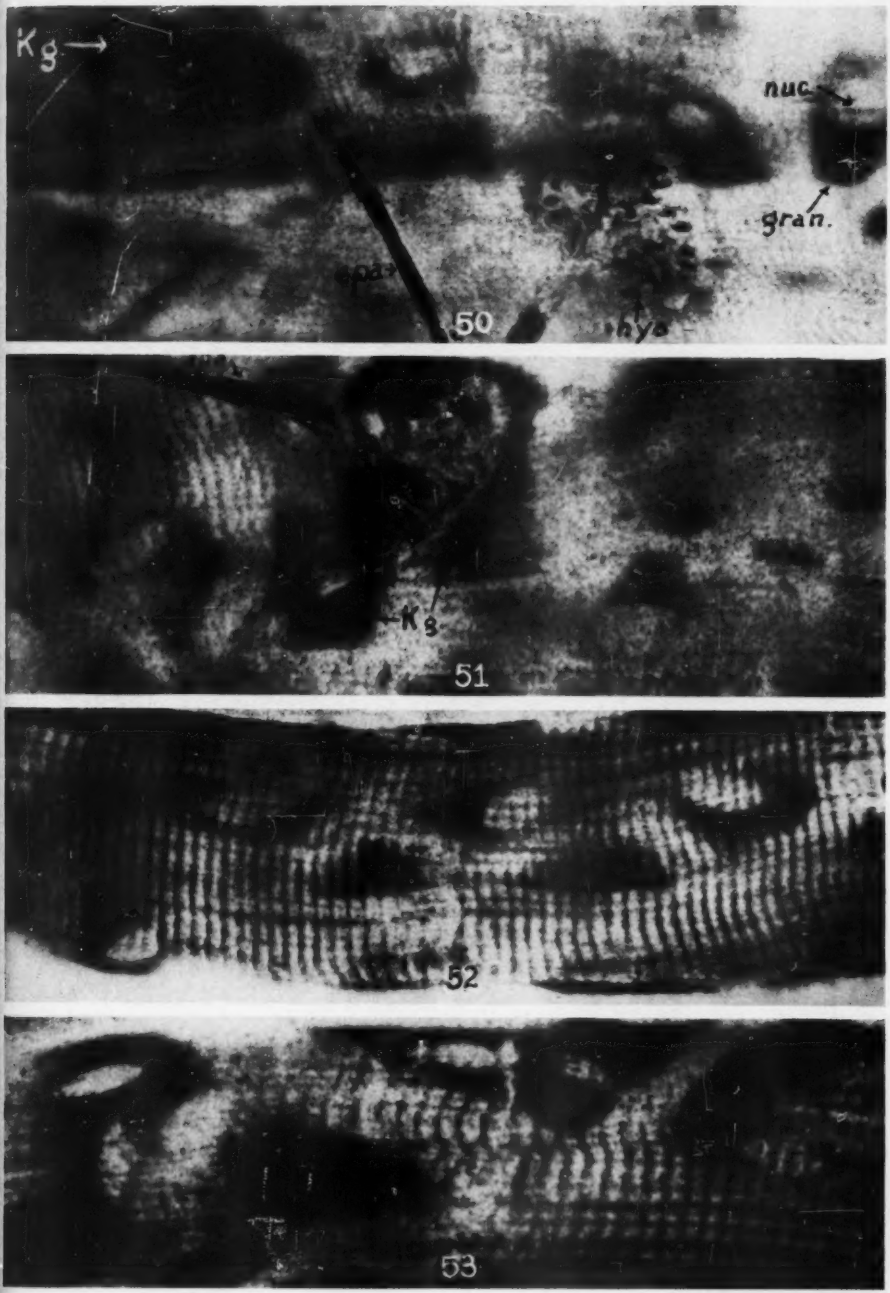
46
Kg
Kg
epa
epa
47
epa
hya
48
epa
hya
49

PLATE 260

FIGS. 50 to 53. Motor end-plates from the gastrocnemius muscle of the rat 72 hours after the degenerative cut of the sciatic nerve. Progressive stages are demonstrated in the direct transformation of the hypolemmal axons of the motor end-plates into granules (Figs. 50 and 51). Practically all of the end arborizations of the motor end-plate (Fig. 51) have undergone granular transformation. The dark islands with a strong affinity for gold in this end-plate represent aggregations of granules discharged from the hypolemmal axons by their direct granular transformation. The clear, rounded, and oval spaces both within the region of the end-plate and out in the myoplasm are occupied by nuclei. Each nucleus has a clear, oval, or rounded space, and is surrounded by a rim of granules that varies in thickness and density. The subsarcolemmal nuclei have become larger than those within the granular sole plate. The increase in granules around the bloated nuclei of the myoplasm may be correlated with a temporary increase of the enzyme, cholinesterase, because of failure of neutralization due to diminution of nondiffusible acetylcholine at this time. The nuclei of the sole plate appear atrophic in comparison with those in the myoplasm of the same fiber. They are either fusiform, rounded, or oval, and with the gold technic form clear spaces surrounded by granules. The granular material both in the sole plate of Kühne and out into the myoplasm is contributed from two sources: the granular transformation of the hypolemmal axons of the motor end-plate, and the nuclei of the sole plate and the sarcolemmal nuclei of the muscle (Figs. 52 and 53). × 850.


Kg
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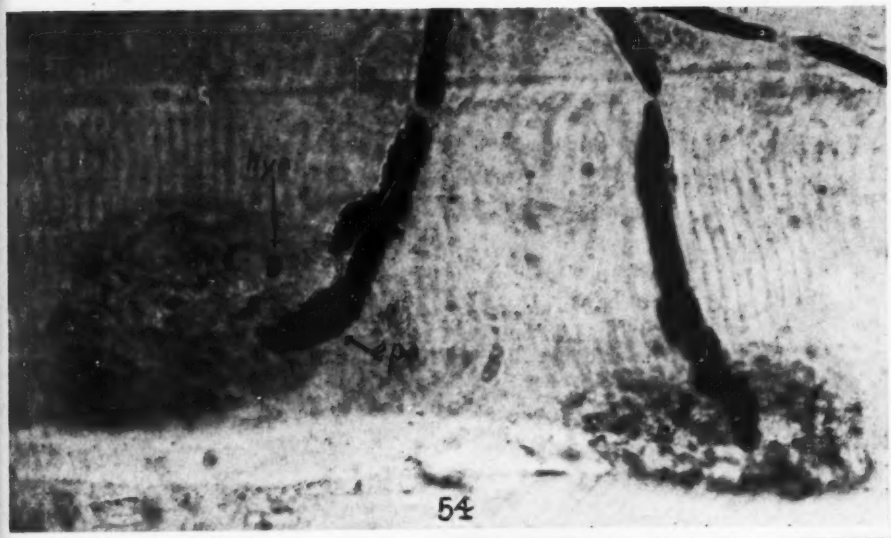
epd?
50
hyp
Kg
51
52
53

PLATE 261

FIGS. 54 to 57. Motor end-plates in the gastrocnemius muscles of rats 72 hours (Figs. 54 and 55), 5 days (Fig. 56), and 20 days (Fig. 57), respectively, after the degenerative cut of the sciatic nerves. Progressive stages are demonstrated of the direct transformation of the hypolemmal axons of the motor end-plates into granules (Figs. 54 and 55). At the fifth day, in many places, the granules of the hypolemmal axons and of the sole plate of Kühne have disappeared by diffusion into the myoplasm (Fig. 56). This depletion of the epilemmal axons of their motor end-plates does not remain constant during the process of wallerian degeneration of the neuromuscular apparatus. At periodic intervals the epilemmal axon discharges the degenerated material into the region of the degenerated motor end-plates. The nuclei of the motor end-plate and sole plate of Kühne become increasingly visible and aggregated due to the accumulation of unused granules around their periphery. Some of these granules are the products of nuclear activity and other granules in this region are discharged from the degenerating epilemmal axon into the region of the degenerated hypolemmal axons. With complete exhaustion of the degenerated material in the epilemmal axons the nuclei still remain visible by the gold technic as clear, rounded, or oval spaces surrounded by granules discharged by the nuclear activity. × 850.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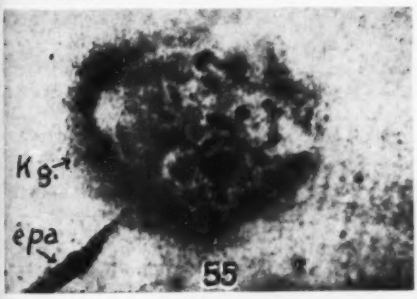
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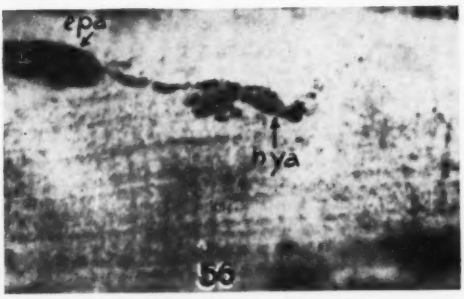
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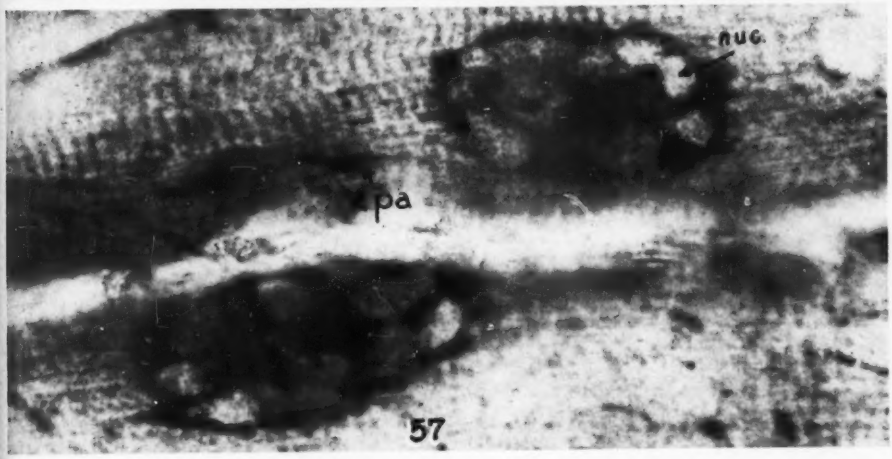
nuc.
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57

PLATE 262

FIGS. 58 to 62. Motor end-plates in the gastrocnemius muscles of rats 5 days (Figs. 58 and 59) and 10 days (Figs. 60, 61, and 62), respectively, after the degenerative cut of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut sciatic nerve for 30 seconds at the rate of 5 per second. The degenerated axonic and myelin materials discharged into the zone of the degenerated motor end-plates have a pleomorphic arrangement: unipolar (Figs. 58 and 59), bipolar (Fig. 61), and multipolar or completely circumferential (Figs. 60 and 62). This gold-impregnated material has been discharged into and around the degenerated motor end-plates in a centrifugal direction from the epilemmal axons. There is a progressive depletion of the material with an affinity for gold from the epilemmal axons during this period of discharge into the region of the degenerated motor end-plates. There is unimpeachable evidence that the gold-impregnated material is initially in direct anatomic continuity with the structures that innervate the muscle. In one end-plate (Fig. 59) the discharged neurosome is in direct anatomic continuity with the hypolemmal axon of the motor end-plate. It forms what has been designated by some neuroanatomists as an ultraterminal nonmedullated branch of the motor end-plate that ends in the same muscle fiber in an enlarged spherical or oblong terminal. This ultraterminal branch of the motor end-plate, however, is merely the initial phase of the discharge of materials from the nerve terminal when anatomic continuity is still maintained. It is the product of the abnormal discharge of substances by abnormal stimulation from the motor ending and not a specific type of morphologic ending as claimed by some observers. × 850.

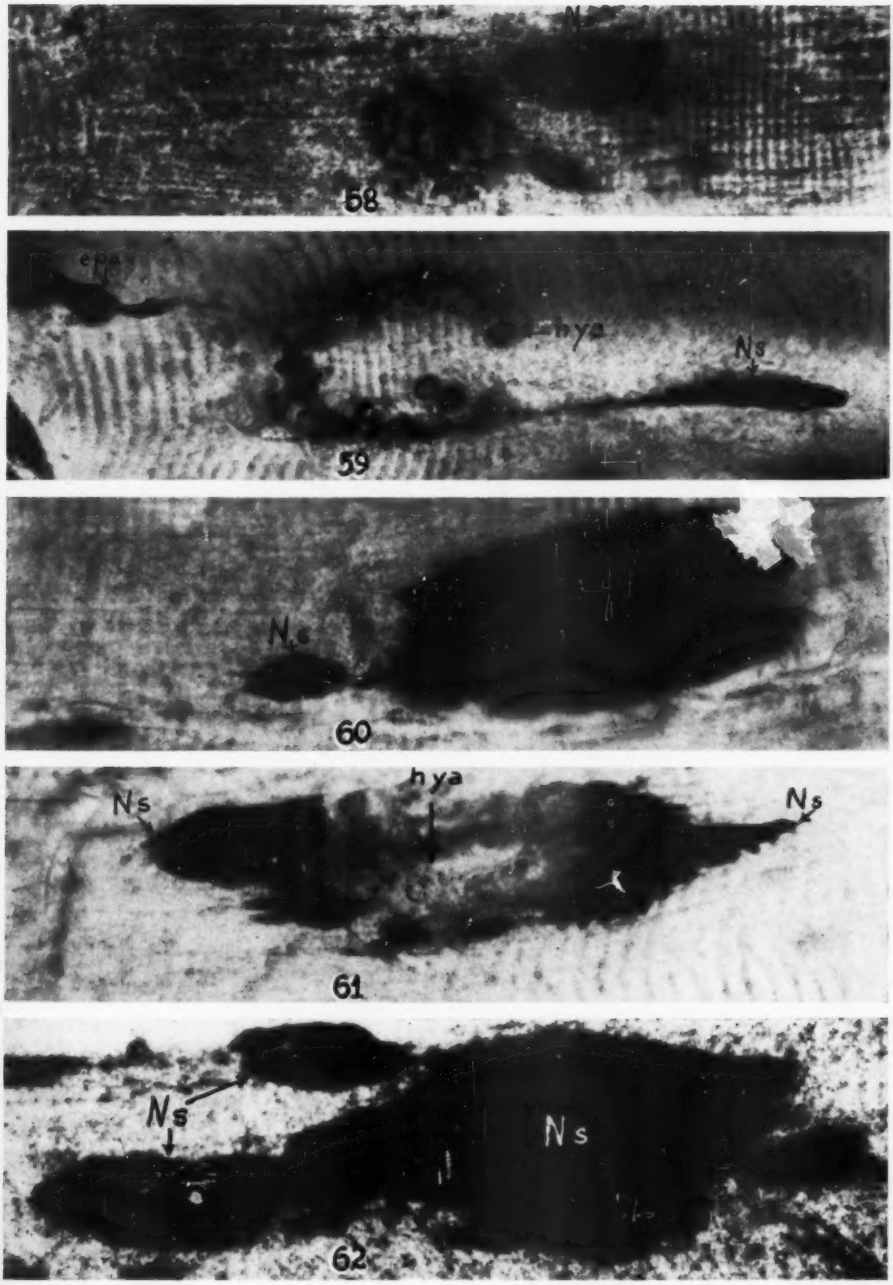
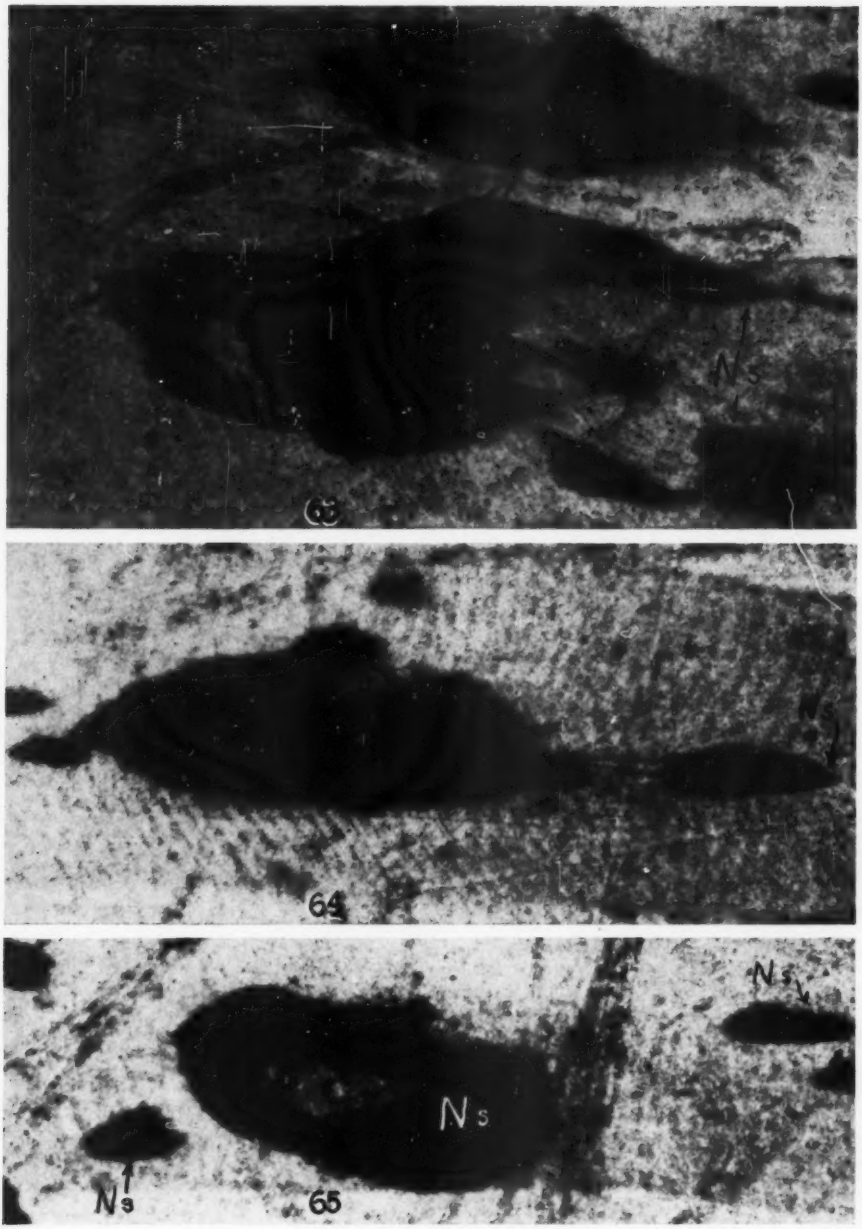

58
epe
hya
Ns
59
Ns
60
Ns
hya
Ns
61
Ns
Ns
62

PLATE 263

FIGS. 63, 64, and 65. Motor end-plates in the gastrocnemius muscle of the rat 5 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. This gold-impregnated material has been discharged into and around the degenerated motor end-plates in a centrifugal direction from the epilemmal axons. The central region of the degenerated motor end-plates may be clear, surrounded by a thick rim of material that has a strong affinity for gold (Fig. 65), or there may be a dark center with radiating dark streaks like a wheel with hub and spokes, constituting the arrangement of the material discharged from the terminal of the depleted epilemmal axon (Fig. 63). To the right of one end-plate (Fig. 64) there is direct anatomic continuity between the gold-impregnated material surrounding the degenerating end-plate and the discharge of a fusiform neurosome out into the myoplasm of the muscle fiber. × 850.



Carey and Associates Structure of Nerve Impulse Transmitter

Plate 264

Figs. 66 to 70. Degenerated motor end-plates in the gastrocnemius muscle of the rat 15 days (Figs. 66 to 68) after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. Curare and electrical stimulation were not used in the animal from which the motor end-plates were obtained (Figs. 69 and 70) 15 days after the degenerative cut of the sciatic nerve. It is clearly evident that under the conditions of the experiment there was an accelerated discharge of increased quantities of substances with an affinity for gold from the epilemmal axons into and about the regions of the degenerated motor end-plates (Figs. 66 to 68) in comparison with the condition in the end-plates after simple section of the sciatic nerve (Figs. 69 and 70). Thus, by experimental means, there may be an acceleration of the discharge of abnormal neurogenic substances from the motor nerve endings into the muscle fiber. × 850.

A

C

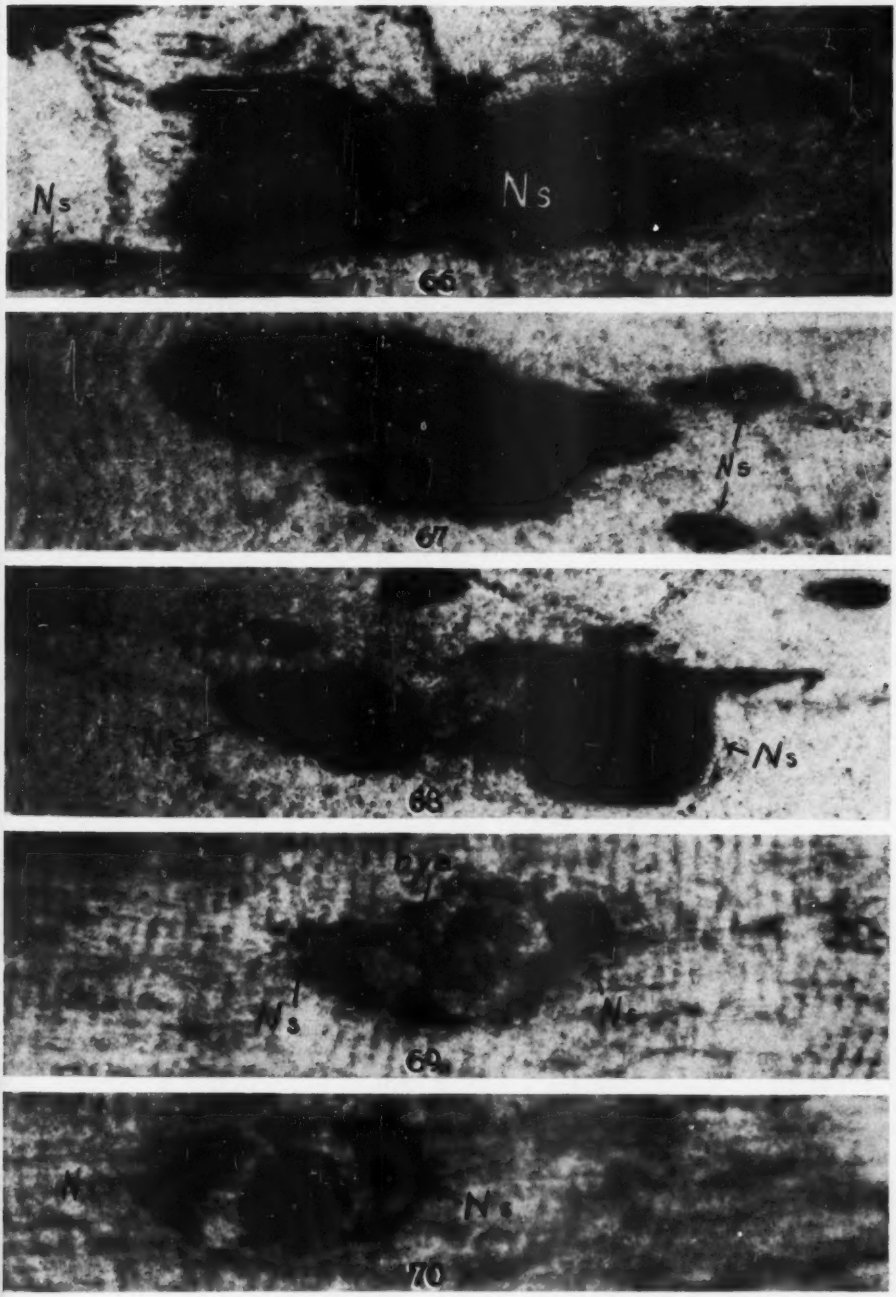

Ns
Ns
66
Ns
67
Ns
68
69
70

PLATE 265

FIGS. 71 to 75. Gastrocnemius muscle fibers from an etherized rat 15 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. The discharged neurosomes (Ns) found in the myoplasm are pleomorphic and hyperchromatic for gold in comparison with the cross striations of the related muscle fibers in which they are found. These neurosomes may be fusiform (Figs. 71 and 72), irregularly oblong with one end tapering (Figs. 73 and 74), arrow-headed in shape, or like rounded droplets that vary in size (Fig. 75). The droplets form series of either single drops widely separated from one another or a closely related series composed of 2 to 10 droplets. The droplets in the middle of the series are usually larger than the terminal ones. Such series of droplets form an irregular fusiform structure separated by clear spaces. The large fusiform neurosomes may have edges that are either serrated (Fig. 71) or festooned (Fig. 72). The sharp projections arranged like saw teeth around the edges of the neurosome may or may not be in direct alignment with the dark cross striations of the muscle fiber. These neurosomes undergo a granular dissolution, and the granules become incorporated into the myoplasm, and are then aligned with the cross striations of the muscle fiber. These neurosomes are periodically discharged from the motor endplates into the muscle fiber. During degeneration they are more persistent than those produced by the artificial overstimulation of normal muscle or those produced by neurogenic shock. × 850.

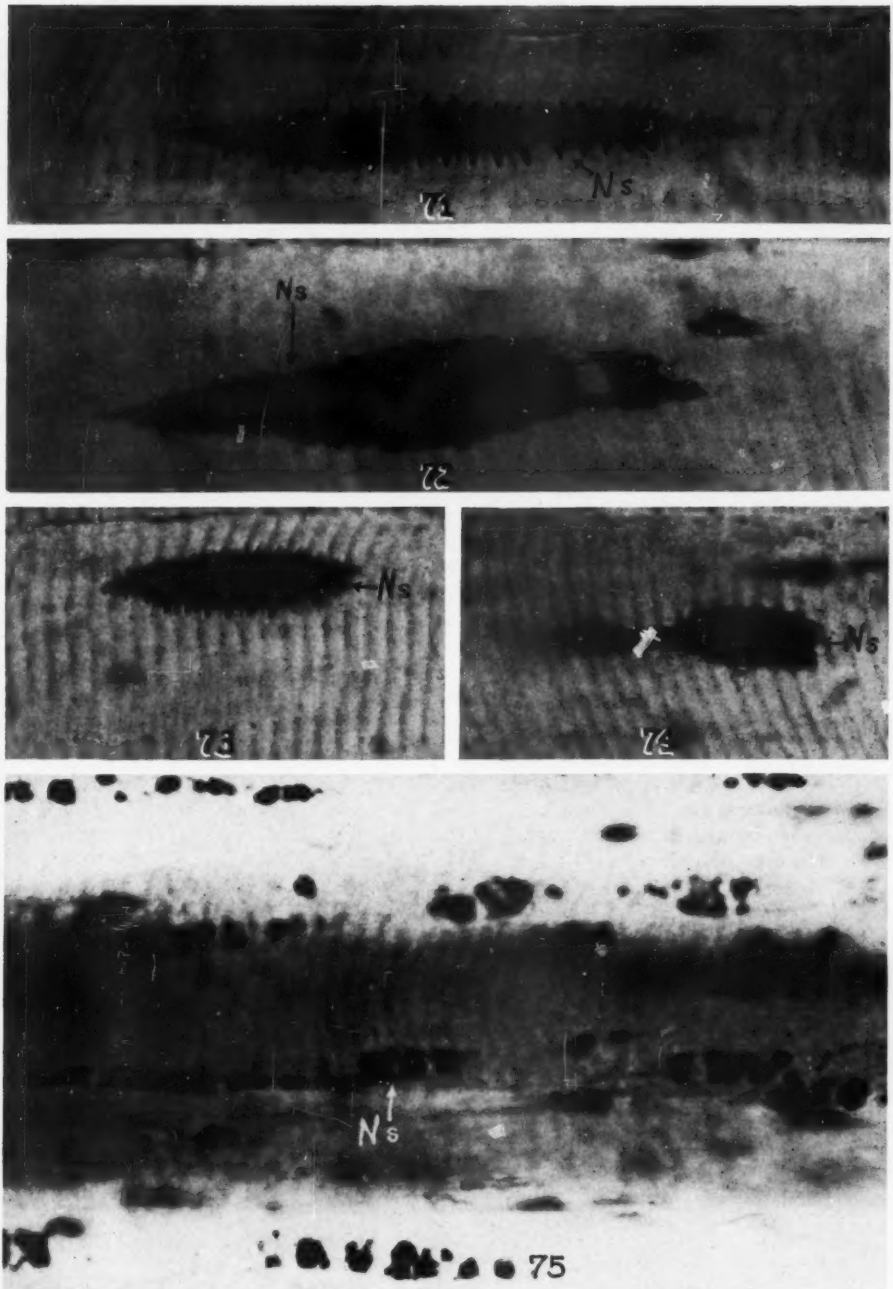

Ns
Ns
Ns
Ns
Ns
75

PLATE 266

FIGS. 76, 77, and 78. Gastrocnemius muscle fibers from an etherized rat 14 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. In a few locations giant muscle fibers (Gmf., Fig. 76) are found. Their diameter is increased three to four times above that of the closely related muscle fibers. These giant muscle fibers are densely impregnated with gold, indicating an abnormal discharge and accumulation of increased quantities of abnormal axonic material in the fiber under the conditions of the experiment. The cross striations of the giant muscle fiber are seen only at its edges because of the dense opacity produced by the discharged nervous material. There is a definite streamlined effect produced by certain large neurosomes (Ns) upon the cross striations of the muscle fiber. This streaming effect of the neurosomes discharged into the myoplasm is detected by the altered arrangement of the cross striations in the muscle fiber (Fig. 77). Certain large muscle fibers (Fig. 78) are not so densely packed with neurosomes as those of the giant muscle fibers (Fig. 76). The neurosomes are clearly evident as gold-impregnated bodies intermingled with the cross striations. In some locations these neurosomes are dense and opaque, while in other places, where they are undergoing granular liquefaction, they are light and cross-striated in appearance. Some of the muscle fibers in close proximity to those containing neurosomes do not possess these bodies in their myoplasm (Fig. 78). The muscle fibers without discharged neurosomes are more lightly impregnated with gold than those that possess them. Figure 76, × 200; Figure 77, ×400; Figure 78, × 850.

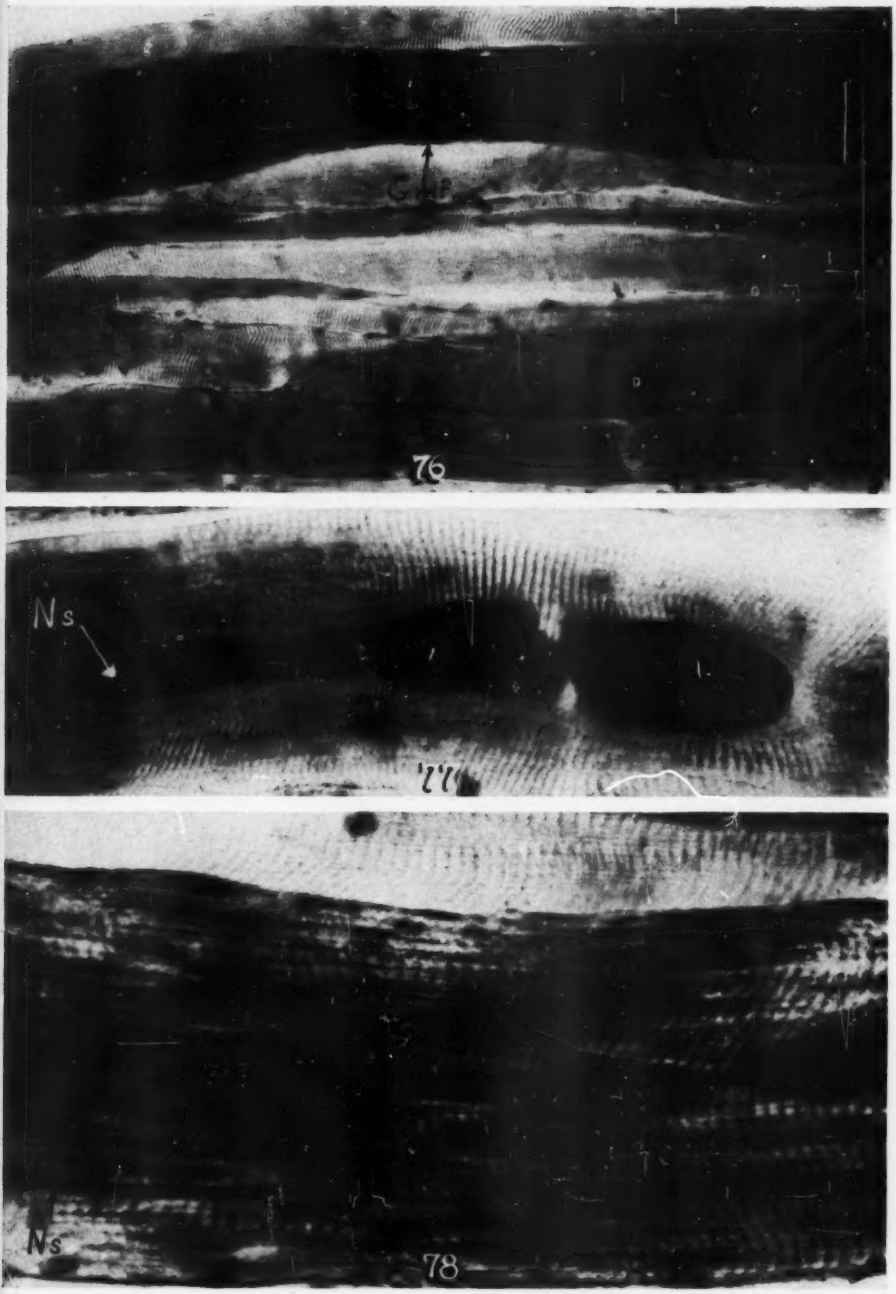

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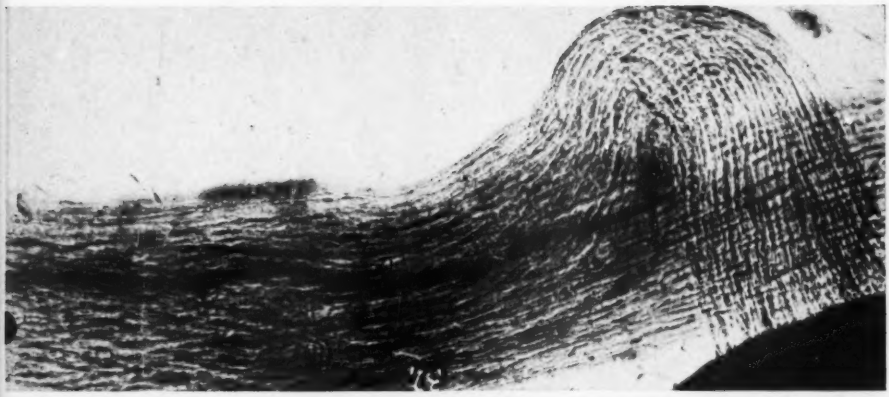
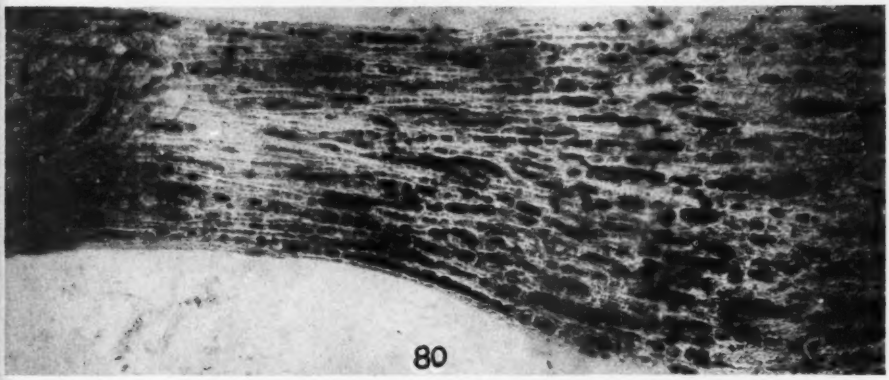
PLATE 267

FIGS. 79 to 82. Branches of the distal stump of the sciatic nerve innervating the gastrocnemius muscle 14 days after the degenerative cut of the nerve. The progressive degeneration of the nerve from its proximal to its distal end is evident. The branch of the nerve just before entrance into the gastrocnemius muscle is completely depleted of the degenerated axonic and myelin substances that have an affinity for gold (Fig. 79). At a slightly more distal point in the same nerve, the gold-impregnated material is found in variable amounts in different axis-cylinders which are fragmented into rounded, oval, or fusiform bodies with a strong affinity for gold (Figs. 80 and 81). Just before the nerve gives rise to branches that directly innervate the muscle, there is again a depletion from the axis-cylinders of material that has a strong affinity for gold (Fig. 82). Only in widely scattered areas are five bodies found that have an affinity for gold. The epilemmal axons of a cut nerve that innervate the muscle are periodically engorged and depleted of the degenerated and fragmented axonic and myelin materials. This rhythmic discharge of degenerated nervous material into the muscle continues in a progressive and periodic manner and in a centrifugal direction until all of the degenerated material is discharged centrifugally into the muscle. × 250.

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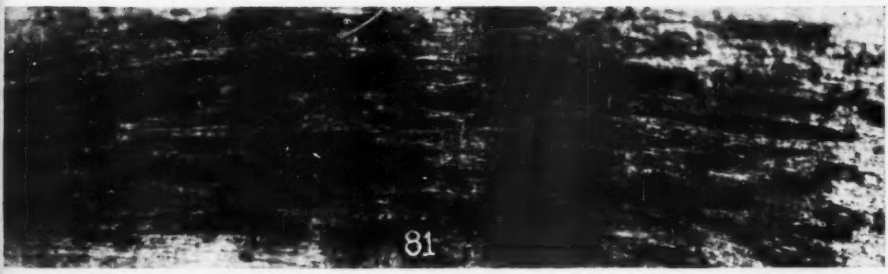
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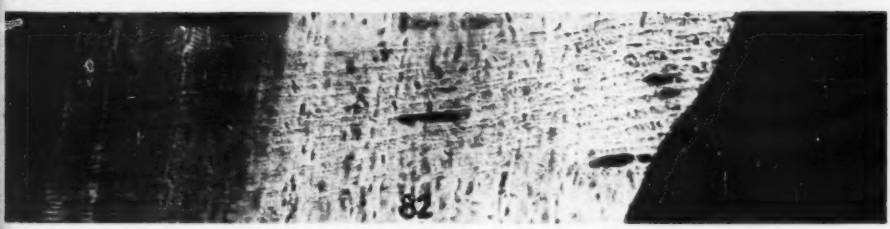
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EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

XIV. Experimental Atheromatosis in Macacus rhesus Monkeys *

W. C. Hueper, M.D.

(From the Warner Institute for Therapeutic Research, New York 11, N.Y.)

Little information is available on the occurrence of "spontaneous" arteriosclerosis in primates. Fox [1] found arteriosclerotic lesions of atheromatous or sclerotic type in only 8 of 796 monkeys studied, and concluded that arterial disease is not a prominent process in the species used. None of the affected animals was a rhesus macaque so commonly used in laboratory work. Kawamura's efforts [2] to produce cholesterol atheromatosis experimentally in 3 monkeys of the species *Macacus fuscatus* gave a negative result. One of these monkeys received 2 egg yolks daily for 150 days, while the other 2 were fed a total of 1,350 gm. and 1,650 gm. of anhydrous wool fat, respectively, within 300 days. Corwin [3] noted in 1938 that all attempts to elicit the condition known as cholesterol arteriosclerosis in monkeys were unsuccessful.

In a critical evaluation of these observations consideration must be given to the fact that monkeys in general are herbivorous animals, which, under normal dietary conditions, show a low tendency toward arterial cholesterol deposition. Only rabbits, when exposed over prolonged periods to an abnormal and excessive intake of cholesterol causing a considerable rise in the level of their blood cholesterol, have so far developed a cholesterol atheromatosis (Hueper [4]). Ssolowjew [5] reported, moreover, the occurrence of lipoid deposits in the aortic intima and media of suckling rabbits, which he attributed to the exclusive milk diet consumed by the rabbits during their early life and which he compared with the lipoid spots often seen in human babies for the same reason.

In view of the fact that primates are phylogenetically closer to man than any other species and that *Macacus rhesus* monkeys are extensively used in laboratory work, it seemed to be pertinent to attempt once more to produce atheromatosis in these animals, utilizing the observations made in suckling rabbits in the experimental conditions to be employed.

Experimental Procedure

Two young *Macacus rhesus* monkeys, born in the Warner Institute for Therapeutic Research, were used in the experiments. One was 5 months old and the second was 6 months old at the start of the experiment. Both had just been weaned and had been placed on a diet

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consisting of carrots, apples, oranges, and bread, with a daily supplement of approximately 90 cc. of milk into which one fresh egg was beaten and which was divided between the two monkeys. The male monkey, which weighed 1.4 kg. at the start of the experiment, in addition received daily by mouth 10 cc. of a 2.5 per cent solution of cholesterol in Mazola oil. The solution was injected into an orange or soaked into bread so as to make it more palatable. After 18 weeks on this management the daily dose of 2.5 per cent cholesterol in oil was raised to 15 cc. This dose was maintained for 20 months, when the animal at the age of 2 years and 5 months was sacrificed by the intracardial injection of 20 cc. of a 4 per cent formaldehyde solution.

The female monkey, which weighed 900 gm. at the start of the experiment, was given 5 cc. of cholesterol-oil daily for a period of 5 months. The dose was then raised to 10 cc. and maintained at this level for the remaining 8 months, at the end of which time the animal, when 18 months old, was sacrificed in the manner described. Both monkeys showed a steady gain in weight during the observation period. The male monkey weighed, at the end, 3.1 kg., whereas the female monkey weighed 1.4 kg. Their appetites were good throughout and they were always lively and playful while under the special dietary regime.

The autopsies showed essentially normal organs in both animals. The histological examination of the internal organs (lung, heart, liver, spleen, pancreas, adrenal, kidney, testis, thyroid) did not reveal any abnormalities. The inferior vena cava of the female monkey had a small area in which the endothelial cells were swollen and had proliferated. The aorta of this animal exhibited similar endothelial changes in the thoracic portion (Fig. 1). In the male monkey similar reactions were more extensive and more widely distributed in the aorta. Several transverse sections showed a crowding of the endothelial cells which were swollen in places and cuboidal. In one level of the ascending part, the endothelial cells were forming a stratified coat of slender cylindrical cells (Fig. 2). The aortic branches and the large and small arteries of the parenchymatous organs were normal.

The histological examination of the internal organs, the aorta and its branches of a monkey 7 months old, continuously nursed by its mother for the entire length of its life and succumbing to pneumonia, did not reveal any abnormal vascular reactions of the type described.

Comment

The observations reported indicate that *Macacus rhesus* monkeys do not readily react to a prolonged nutritional intake of excessive amounts of cholesterol with the formation of atheromatous aortic

lesions, even when exposed to such a dietary regimen during an early period of life. The endothelial aortic lesions, however, may represent proliferative responses to the cholesterol ingested, since similar endothelial reactions have been found in dogs following the repeated intravenous injection of other atheromatogenic substances, such as hydroxyethylcellulose solution (Hueper [6]) and after a prolonged oral administration of excessive amounts of cholesterol (Hueper[7]). They may thus represent early atheromatous reactions.

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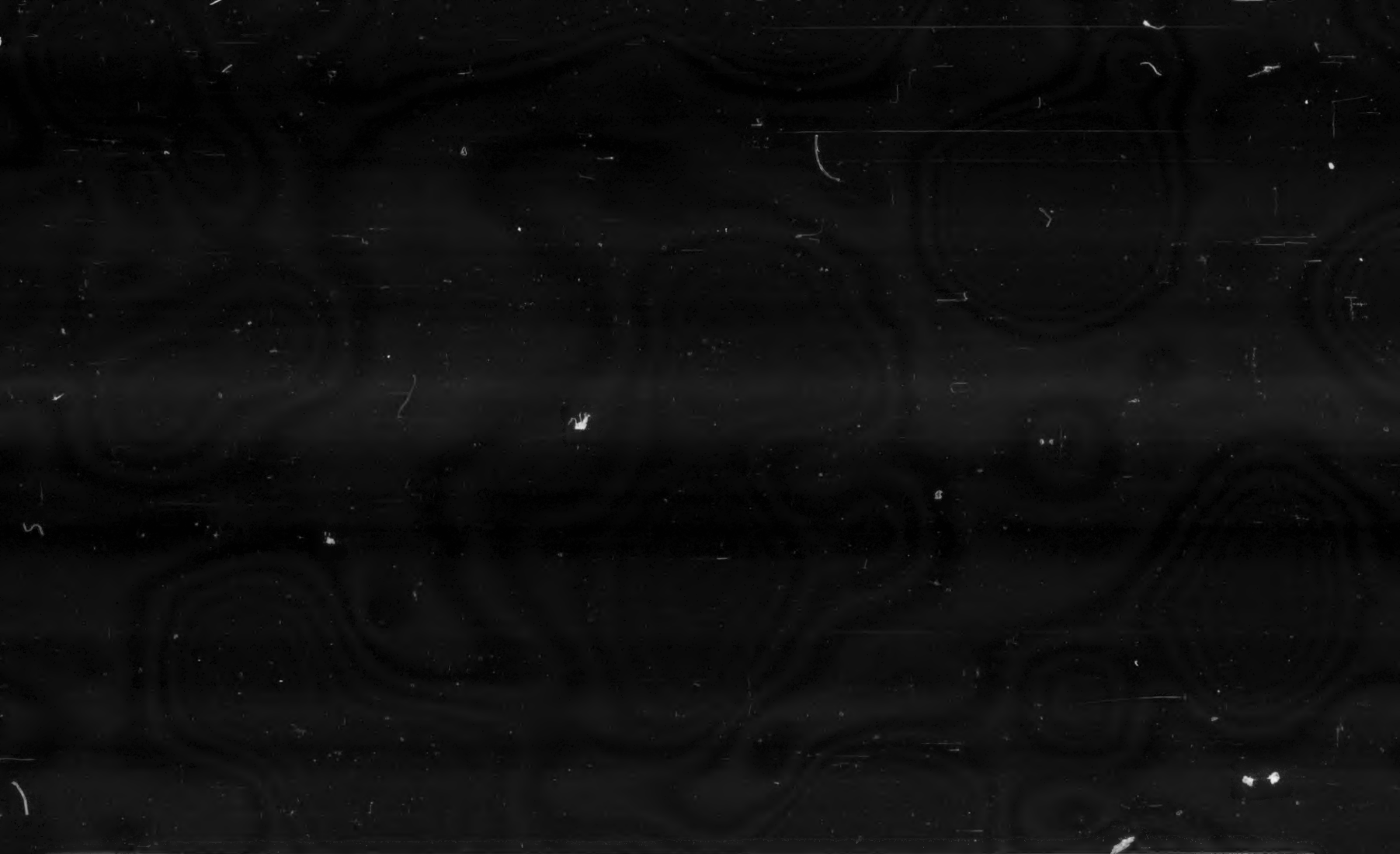
[*Illustrations follow*]

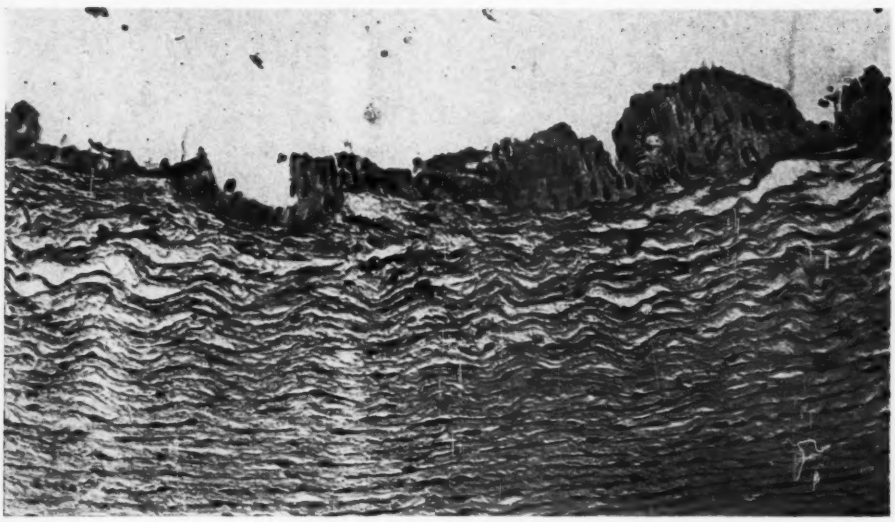
DESCRIPTION OF PLATE

PLATE 268

FIG. 1. Aorta with proliferated and swollen endothelial cells.

FIG. 2. Aorta covered by a cushion of stratified, slender endothelial cells.





METASTATIC CALCIFICATION ASSOCIATED WITH HYPERVITAMINOSIS D AND HALIPHAGIA *

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The occurrence of metastatic calcification with various types of osseous lesions [1-5] and with chronic renal disease [6] in human pathology is well known. Experimentally, the feeding of calcium salts,[7,8] the administration of large doses of vitamin D,[9-11] and the injection of great amounts of parathyroid hormone [12,13] have also resulted in metastatic calcification. From the point of view of human pathology the rôle of vitamin D in the production of this lesion rests on rather scanty evidence.[14-17]

HUMAN CASES OF HYPERVITAMINOSIS D FROM THE LITERATURE

Putschar [14] observed a male infant, 5½ months old, who received vigantol (irradiated ergosterol) for about 3 months before death. Autopsy disclosed emaciation, small adrenal glands, moderate fatty metamorphosis of the liver, a peculiar lipogranulomatous reaction in the hypodermis, and calcium deposits in the kidneys. These deposits involved mainly the epithelial cells, lumina, and basement membranes of the distal convoluted tubules, and also the stroma adjacent to them. The collecting tubules and the surrounding stroma in the pyramids were similarly involved and in much greater degree. No reactive or regressive changes were present near the calcium deposits.

Thatcher [15] reported the case of a male infant, 18 months old, who received irradiated ergosterol for 9 months before death. At autopsy the kidneys were enlarged, firm, and pale yellow. The medulla at the bases of the pyramids showed tiny, gritty, gray particles which microscopically were masses of calcium lying mainly in the lumina of the collecting tubules at the corticomedullary junction, with calcification of some adjacent epithelial lining cells. Calcified cells were identified in the masses of calcium, some of which were encircled by cellular fibrous tissue. The segments of tubules proximal to the calcium masses were dilated. Calcium was not found in other organs. The liver showed extensive fatty metamorphosis.

Thatcher [16] reported a second case in a male infant, 11½ months old, who had received much solar irradiation, two ultraviolet ray treatments, and a large amount of cod-liver oil for 4 months. Necropsy findings were practically identical with those in his first case.

Gissel and Bufe [17] administered large doses of dihydrotachysterol

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to two infants with meningomyelocele and hydrocephalus. One died at the age of 128 days. In addition to ascending meningitis and pyocephalus found at autopsy, the kidneys showed heavy calcium deposits in the epithelium of the cortical and medullary tubules as well as calcium casts in their lumina and scattered, finely granular calcium incrustations in the neighboring interstitial tissue. The second infant died of ascending meningitis and internal pyocephalus at the age of 54 days. The kidneys showed detached calcium casts in the cortical portions of the collecting tubules with absent or newly regenerated epithelial cells adjacent. The lungs, heart, liver, spleen, and stomach in both cases showed no calcium deposits.

Wells and Holley [5] thought that the reasons for metastatic calcification in the lungs, left atrial endocardium, kidneys, gastric mucosa, and hypodermis in their case of osteitis deformans were the easier mobilization of calcium from the bones in this disease, and the massive doses of viosterol given to the patient.

The observation of a recent case of metastatic calcification, in which the patient ingested vitamin D and several inorganic salts, furnishes provoking problems for solution.

Report of Case

A cab driver, 44 years old, born in Scotland, was admitted to St. Luke's Hospital in Denver under the care of Dr. C. R. Cooper, who kindly gave permission for use of the clinical details. Since the patient was comatose, the history was obtained from his wife, who stated that for about 6 months before admission and for little understood reasons he had been taking daily doses of a vitamin D preparation as well as liberal amounts of a mildly laxative compound containing sodium sulfate, sodium bicarbonate, sodium phosphate, and sodium chloride. His wife warned him repeatedly that this was a hazardous procedure without the advice of a physician, but he persisted in his autotherapy. Two weeks before admission he had suffered from malaise, weakness, restlessness, and drowsiness. One week before he had seen an osteopath who diagnosed Bright's disease and prescribed some unknown medication. Since he did not improve and experienced periods of stupor alternating with bouts of delirium, he was admitted to the hospital on July 2, 1944, at 11:35 A.M. His temperature was 99.8° F. and his pulse was 90 beats per minute. His respirations were labored and numbered 26 per minute. Physical examination revealed stupor, pallor, a loud, blowing, systolic murmur over the entire precordium, lungs clear to percussion and auscultation, and no significant changes in the abdomen except for a healed appendectomy scar. The voided urine was amber, murky, and acid. The specific gravity was 1.022, albumin was 3 plus, and sugar and acetone were absent. Microscopic examination disclosed many granular casts, an occasional leukocyte, and 20 to 30 erythrocytes per low-power field. A roentgenogram of the chest showed a diffuse fine mottling throughout both lungs with some hilar mottling. This appearance was interpreted as pulmonary edema. Blood counts and chemical determinations were not obtained. At 4:00 P.M., when 500 cc. of plasma and 1000 cc. of normal saline solution were injected intravenously, the pulse was rapid, irregular, and difficult to count. At 7:00 P.M. the patient was expectorating thick, yellow sputum, his breathing was stertorous and rapid, and he moved restlessly

in bed, although in coma. His pulse was 140 and his temperature was 98° F. At 1:30 A.M. on July 3rd, he died.

AUTOPSY FINDINGS

At autopsy, 8 hours after death, the body weighed 160 lbs. (78 kg.) and measured 72 inches (183 cm.) long. Externally, the only significant finding was a healed appendectomy scar. The panniculus adiposus was 3 cm. thick and the skeletal muscles were well developed and dark red. The peritoneal surface was smooth and moist except for adhesions of the omentum and the cecum to the under surface of the laparotomy scar. The pleural cavities and the pericardial sac were normal. The thymus was small and consisted of soft, yellow tissue. The thyroid gland *in situ* was normal in size, firm, gray-red, and finely lobulated. No tumors were found near the thyroid gland, although the parathyroid glands were not examined specifically.

The heart weighed 480 gm. and the epicardial fat was abundant. The myocardium was firm and gray-red. The right ventricle was 3 to 5 mm. and the left ventricle 16 to 20 mm. thick. A gray-red clot adhered to the lining of the left auricular appendage. The foramen ovale was closed. The valve leaflets were all thin and translucent. The valve circumferences were as follows: tricuspid, 12 cm.; pulmonic, 6.8 cm.; mitral, 10 cm.; and aortic, 6.5 cm. The coronary arteries were patent and their elasticity was moderately reduced. The first portions of the main branches showed irregular, yellow, intimal thickening. The aorta was moderately elastic and of usual caliber. A few pin-point, yellow dots marked the intima at the root and fine, yellow, longitudinal streakings were lightly scattered in the intima of the abdominal segment. The stems of the great vessels were patent.

The right lung weighed 780 gm.; the left lung, 810 gm. The surfaces were smooth and the lobes were discrete. Except for the apices and anterior margins, both lungs were subcrepitant on sectioning, dark red, finely black-streaked, and oozed plentiful foamy gray fluid. The trachea and bronchi had intact mucosa and a fairly abundant content of pale yellow mucoid material. The pulmonary arteries were normal. The mediastinal lymph nodes were small, soft, black and gray mottled.

The spleen weighed 420 gm. Visible through the thin capsule and extending down into the firm, flat, gray-red parenchyma were four dark purple, map-like areas, 1.5 to 4 cm. in greatest diameters.

The esophagus was normal. The stomach contained a scant amount of yellow, thin fluid and was lined by a reddened, well folded, intact mucosa. The small intestine contained similar fluid. The appendix was absent. The colon contained soft, well formed, dark brown feces.

The liver weighed 2,280 gm. The capsule was thin. The cut section was firm, tan, and focally marked by pale yellow areas, 6 to 12 mm. in diameter. The biliary ducts and blood vessels were well preserved. The gallbladder had a smooth serosa, a fairly thin wall, a dark green mucosa, and contained one large, oval stone measuring 4.5 by 3.5 by 2.5 cm., which was rough, tan and dark green, and six dark green, faceted calculi measuring 5 to 6 mm. in diameter. The bile was thick and dark green. The pancreas was normal in size, firm, tan, and coarsely lobulated.

The cortices of the large adrenal glands were fairly thick. The medulla of the right was entirely, and that of the left partly, liquefied.

The right kidney weighed 320 gm.; the left, 330 gm. The capsules stripped easily revealing smooth, swollen surfaces. The cut section (Fig. 1) was firm, flat, pale tan, and unevenly marked by pin-point, dark red dots. The cortices were 5 to 7 mm. thick and the striations were hazy. The pyramids were uniformly enlarged and the striations were fairly distinct. The pelves and ureters were normal. The urinary bladder was empty and the lining was gray-red and wrinkled. The prostate, seminal vesicles, testes and epididymides were grossly not remarkable.

The ribs and vertebrae were hard and difficult to saw. The marrow of the ribs was light tan, soft, and abundant. The cranium was not examined.

Microscopic Examination

The tissues were fixed in 4 per cent formaldehyde, cut at 6 μ from paraffin, and stained with hematoxylin and eosin.

The thyroid showed large follicles well filled with colloid and lined by low cuboidal epithelium. The stroma contained a few small groups of lymphocytes. Calcium was deposited beneath the endothelium of many capillaries and in the intima of several small and medium arteries and veins.

Calcium was deposited in many muscle fibers of the heart (Fig. 2). It was most abundant in the muscle of the septum and left ventricle, moderately plentiful in the left auricle, sparse in the right ventricle, and absent from the right auricle. Subendothelial calcium deposits were observed in blood vessels of all sizes and the degree of deposition paralleled that seen in the myocardium of the four chambers. Much calcium was deposited in the endocardium of the left auricle, the appendage of which was stuffed by loosely adherent ante-mortem thrombus. In the aorta the intima was mildly thickened by lipoids, small groups of foamy macrophages, calcium, fibrous connective tissue, and hyalin. The other coats were intact.

The lungs revealed the following changes: diffuse hyperemia, edema fluid distending most alveolar ducts and alveoli, numerous alveolar phagocytes, dust macrophages in alveoli and in small groups in the stroma, and deposition of calcium (Fig. 3) in abundance beneath the endothelium of veins of all sizes, in many alveolar walls, in the submucosa of many bronchi and bronchioles, and in small amounts in the media of some arteries.

The spleen showed engorged sinusoids, recent sinusoidal hemorrhages, demarcated areas of coagulation necrosis, and calcium deposits in the intima of many large and small arteries and veins. The accessory spleens contained a few blood vessels with slight intimal calcium deposits.

In the upper half of the fundic mucosa of the stomach the glands were greatly autolyzed. The lower half showed extensive deposits of calcium in and around glandular crypts (Fig. 4) with involvement of both interstitial tissue and gland cells.

The parenchymal cells of the liver showed the finely foamy appearance of abundant glycogen. A few groups of liver cells contained coarse cytoplasmic fat vacuoles. The blood vessels were well preserved and contained no calcium. The periportal areas and the biliary ducts were intact. The perimuscular coat of the gallbladder revealed increased fibrous connective tissue and infiltrations of lymphocytes.

In the pancreas, calcium was deposited beneath the endothelium of blood vessels of all sizes, including interacinar and islet capillaries. Calcium was present also in the lamina propria of several medium and large ducts. An area of acinar tissue and fat tissue was partly liquefied and infiltrated by many segmented neutrophils and monocytes. Several groups of acini and some islets were autolyzed.

The adrenal glands displayed no significant histologic abnormalities.

In the kidneys (Fig. 5) numerous masses of calcium were found in all segments of the tubular system of the nephrons and were intermingled with sloughed calcified epithelial cells in the lumina of the tubules. Hyaline droplets distended many epithelial cells lining the convoluted tubules. Recently formed thrombi, some focally organized, plugged many medium and large veins. Many lymphocytes infiltrated the interstitial tissue. Calcium was deposited beneath the endothelium of some large arteries. Granules and masses of calcium were seen in the capsular fluid of some glomeruli and foreign body giant cells surrounded a few masses of calcium in the tubules. Hyaline casts filled many tubules and leukocytic casts distended others. A few glomeruli were obliterated by fibrosis, but most were well preserved.

The urinary bladder was not remarkable.

The prostate contained a few groups of moderately dilated acini. In the peripheral part of the stroma some large arteries exhibited deposits of subendothelial calcium. A few spermia were present in the tubules of the testes. The seminal epithelium was developed through the spermatid stage. An occasional tubule was obliterated by fibrous connective tissue.

A section of rib (Fig. 6) revealed a marrow-cell/fat-cell ratio of 75/25, fairly numerous megakaryocytes, a great increase in the myeloid/erythroid ratio, and a definite shift to the left in the neutrophilic granulocyte line. The cortical and medullary bone was involved by extensive ragged fraying and thinning of the bony trabeculae with countless osteoclasts nestling in the recesses along the scalloped edges of the bony trabeculae. Giemsa staining confirmed these findings.

The final anatomic diagnoses were as follows: Metastatic calcification, on the basis of hypervitaminosis D and haliphagia (ἅλς, *salt*, + φαγεῖν, to eat), involving the heart (chiefly of the endocardium and myocardium of the left auricle and ventricle), the lungs (pulmonary veins, alveolar walls, and bronchi), the fundic mucosa of the stomach, the kidneys, the pancreatic ducts, and the blood vessels of the thyroid gland, heart, spleen, pancreas, and prostate; calcific and hyaline droplet changes in the renal tubules with chronic interstitial inflammation and venous thrombosis; cardiac hypertrophy (480 gm.); mural thrombosis of the left auricle; pulmonary edema; acute passive congestion of viscera; recent infarcts in the spleen; osteoclastic resorption of bone; myeloid hyperplasia of bone marrow; acute focal pancreatitis; chronic cholecystitis and cholelithiasis; slight fatty metamorphosis of the liver; two accessory spleens; and absence of appendix, fibrous peritonitis, and healed laparotomy scar.

Qualitative Chemical Analysis of Lungs, Kidneys, and Gastric Mucosa

Fragments of lung (mainly alveoli in the periphery), kidneys (cortex and medulla), and fundic mucosa of the stomach were ground separately into a fine mush in a mortar. Microscopic examination of these tissues suspended in distilled water revealed amorphous material and fragmented cells but no crystals. When treated with a 50 per cent solution of hydrochloric acid, none of these tissues showed any macroscopic change. However, when 50 per cent sulfuric acid followed by 50 per cent hydrochloric acid was applied to the lung tissue, a grossly visible, violent evolution of gas bubbles was observed indicating the presence of a carbonate radical. The sulfuric acid evidently acted in some way to release calcium carbonate from the organic matrix of the lung, so that the hydrochloric acid was able to react with the carbonate

to produce carbon dioxide. This reaction was negative with the tissues from the kidney and gastric mucosa. When a 50 per cent solution of sulfuric acid was mixed with all three tissues, abundant formation of calcium sulfate crystals was seen histologically, thus demonstrating the presence of a calcium ion in all of them. When the kidney and gastric mucosa were treated with concentrated nitric acid and ammonium molybdate reagent and then heated, a yellow precipitate was formed indicating the presence of a phosphate radical in these tissues. With the same test the lung tissue was negative, for not only did no precipitate form, but the organic matter in the tissue was completely digested, so that a clear solution resulted. In summary, the qualitative chemical analysis of these organs showed that calcium phosphate was the salt deposited in the kidneys and gastric mucosa and that calcium carbonate was the salt deposited in the lungs.

Discussion

The case described is definitely an example of metastatic calcification as is easily proved by reference to the works of Askanazy,[1] Wells,[2] deSanto,[3] Grayzel and Lederer,[4] Wells and Holley,[5] and Herbert, Miller, and Richardson.[6] The heavy deposition of calcium in the endocardium and myocardium of the left chambers of the heart, in the pulmonary veins, bronchi, and alveolar walls, in the fundic mucosa of the stomach, in the kidneys, and in many arteries, capillaries, and even in veins, has been described by these authors. The places where acids are formed; namely, the lungs, the fundic mucosa of the stomach, and the kidneys, are organs in which the tissues are rendered alkaline when these acids are elaborated so that calcium deposition is favored.[2] The calcification of the endocardium and myocardium of the heart, of the arterial and capillary channels of the systemic circulation, and of the capillary and venous channels of the pulmonary circulation may also be explained on this basis, since the pH of the blood is higher in these tissues than in the venous side of the circulation, especially in the right chambers of the heart and in the pulmonary arteries. The ingestion by the patient of four inorganic salts containing sodium ions was a factor tending to increase tissue alkalinity. The deposition of calcium in the tissues was in the nature of a precipitation in previously healthy tissue cells and stroma, for the only degenerative change was the hyaline droplet alteration of the epithelial cells of the convoluted tubules of the kidneys and the only inflammatory infiltrations of significance were the interstitial infiltrations of lymphocytes and the leukocytic casts in some tubules of the same organs. This is in conformity with the original concept of Virchow,[18] who thought that the calcium

deposits in the stomach and lungs of his cases represented a direct calcification of the tissue by which lime salts penetrated and filled up the constituent parts of the organs involved.

Large doses of vitamin D in various forms have been employed to produce metastatic calcification in animals. Smith and Elvove [9] observed calcium deposits in the thoracic aorta, interalveolar septa, and kidneys of rabbits given irradiated ergosterol. Biochemical analysis revealed a progressive increase of serum calcium and the development of high inorganic phosphorus levels. In this study an elevated serum calcium and an increased serum phosphate level were necessary to produce appreciable tissue calcification, whereas with low or normal serum phosphorus values, calcium was not deposited in the tissues, even though the calcium of the blood was elevated. The authors thought that the salt concerned was calcium phosphate precipitated out in the tissues.

By giving viosterol to rats, Shelling [19] found that a greater susceptibility to hypercalcification of soft tissues resulted when the amount of phosphorus in the diet was increased.

With rats on an alkaline diet and large doses of oral calciferol, Gough, Duguid, and Davies [11] observed a heavy, quantitatively measured deposition of calcium in the kidneys, which was more pronounced than when the animals were on an acid diet. In excretion studies they found that the average urinary phosphorus was relatively low on an alkaline diet as compared to that on an acid diet. They also showed that phosphorus excretion was relatively decreased on both acid and alkaline diets by the calciferol.

In the experiments of Hess, Benjamin, and Gross,[20] the excessive administration of irradiated ergosterol caused a hypercalcemia in dogs fed a diet absolutely free of calcium. They thought that this excess calcium in the blood must come from the bones. The hypercalcemia was greatly reduced by the intravenous injection of sodium bicarbonate, after which a great excess of calcium and phosphorus was found in the lungs and kidneys and the calcium content of both urine and feces was definitely diminished. The histologic reaction in the bones of guinea-pigs given large doses of irradiated ergosterol was studied by Grauer,[21] who found in sections of costochondral junctions and long bones a resorption of bony trabeculae which were abundantly lined by osteoclasts, an increase in the size of the lacunar spaces, a thinning of the cortex, a proliferation of fibrous connective tissue in the marrow, and hemorrhage in the marrow cavities.

By applying the results of these experiments to the findings in the patient described, the vitamin D he ingested could have caused hyper-

calcemia,[9,20] phosphate retention,[9,11] and mobilization of calcium phosphate from the bones [20] as indicated by osteoclastic activity.[21] His diet was definitely alkaline through the ingestion of four sodium salts, which would favor the deposition of calcium salts in the soft tissues such as those of the kidneys.[11] An added source of phosphate further to augment the level of phosphate in the blood was available from the sodium phosphate taken by the patient. Finally, the sodium bicarbonate could have enhanced the calcium retention and favored an increase of calcium and phosphorus in such organs as the lungs and kidneys.[21]

No obvious tumors were observed near the thyroid gland. The absence of osteitis fibrosa cystica militated against the presence of a neoplasm in an aberrant parathyroid gland. The changes in the kidneys of patients with primary hyperparathyroidism have been described by Anderson.[22] The characteristic features include the following: interstitial fibrosis, interstitial, *mainly peritubular,* calcification, interstitial infiltrations of lymphocytes and plasma cells, cystic dilatation of tubules, thickening and sometimes calcification of the basement membranes of tubules, a relative absence of active glomerulitis or involvement of the epithelial cells of the tubules, sometimes formation of calculi and superimposed ascending infection, and obstruction and dilatation of tubules by the peritubular calcium deposits. Not only do these microscopic changes differ strikingly from the histologic features for the kidneys in my case, but the weight of the kidneys in the cases described by Anderson [22] was far below the tremendous weight (650 gm., combined) of the kidneys in the case reported. Both the gross and microscopic findings in the kidneys of the hitherto reported human cases of hypervitaminosis D [14-17] are much more nearly comparable to those in my case.

The mural thrombosis of the left auricle and the thrombosis of the renal veins were possibly on the basis of a high tissue alkalinity associated with a high content of calcium ions in the blood in these locations to hasten the clotting reactions. Askanazy's [1] first case of widespread involvement of the skeletal system by malignant melanoma and hemorrhagic nephritis also showed thrombosis of the renal veins. The cardiac hypertrophy may be explained on the basis of enlargement of uncalcified muscle fibers to compensate for those involved by calcium deposits. The recent infarcts of the spleen could have been caused by lodgment of emboli from the mural thrombus in the left auricle, although these emboli were not actually demonstrated. The deposition of calcium in the lamina propria of the pancreatic ducts has not hitherto been de-

scribed in cases of metastatic calcification. No explanation is obvious for the myeloid hyperplasia of the bone marrow.

Summary

Widespread metastatic calcification involving the heart, lungs, gastric mucosa, kidneys, pancreas, and numerous blood vessels of a male patient, 44 years of age, was associated with the ingestion of vitamin D and alkaline inorganic salts. The reasons for this extensive direct calcification of soft tissues in all probability included hypercalcemia, phosphate retention, mobilization of calcium phosphate from the bones brought about by osteoclastic activity, a high alkaline diet, an excess of ingested phosphate, and the enhancement of calcium retention by the intake of sodium bicarbonate. Qualitative chemical analysis demonstrated the presence of calcium carbonate in the lungs and of calcium phosphate in the gastric mucosa and kidneys.

Since this paper was submitted for publication, a case of metastatic calcification in a woman, 32 years old, has been reported by J. M. Bauer and R. H. Freyberg (Vitamin D intoxication with metastatic calcification. *J. A. M. A.*, 1946, 130, 1208–1215). This woman received large amounts of irradiated ergosterol, largely calciferol, for 1 year before death. At autopsy calcification of the kidneys, right knee joint, myocardium, endocardium of the left auricle, systemic arteries, lungs, dura, and subcutaneous tissue of the left ischial region was found. Also present were a chronic fibrous pneumonitis and a chronic duodenal ulcer.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 269

FIG. 1. Kidney, cut section. About ½ ×.

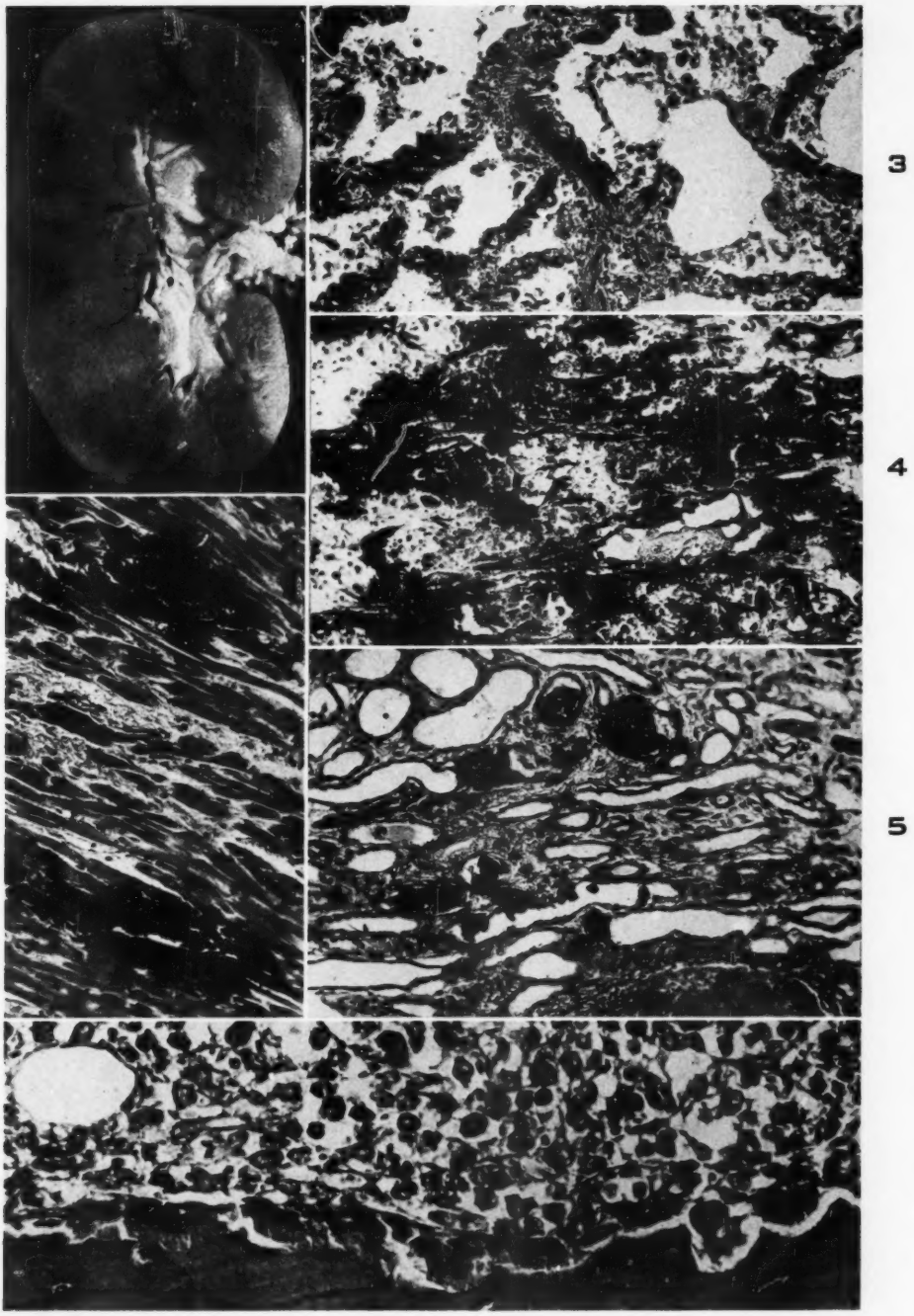
FIG. 2. Myocardium of left ventricle, showing groups of calcified muscle fibers. × 130.

FIG. 3. Lung, showing calcium deposits in walls of alveoli. × 130.

FIG. 4. Gastric mucosa with calcium deposits in gland cells and interstitial tissue. × 135.

FIG. 5. Kidney, showing calcium masses in lumina of tubules, several with calcified cells. Of note is the renal vein in the lower right-hand corner, containing a thrombus. × 130.

FIG. 6. Rib with osteoclasts lining a ragged bony trabecula below, and cellular marrow above. × 365.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PATHOLOGICAL CALCIFICATION IN THE GINGIVAE *

WILLIAM F. BARNFIELD, D.D.S.†
(From the Indiana University School of Dentistry, Indianapolis 2, Ind.)

In a recent paper, Orban [1] has presented a type of gingival inclusion which he termed "dentin-cementum remnant." He believes that these rounded, basophilic, acellular calcified structures have their origin in fragmented dental structures. While Orban's explanation is direct and simple, it is believed that there is another method of formation of calcified gingival bodies. It is the purpose of this paper to show how calcified bodies may develop *in situ* because of local influences other than fragmentation of dental structures.

Material for this study was obtained from the slide files and reserve histological material that has been accumulated over a period of nearly 12 years by the University of Illinois College of Dentistry. Among 120 specimens of human gingivae, 4 showed lesions that were believed to be stages in the development of large and fully calcified bodies of the kind illustrated in Figure 8.

The following criteria were used in deciding which specimens were to be included in this study. (1) In order to exclude superficial débris, only the structures located beneath the epidermis were included. (2) To rule out cementicles, no structure in or close to the periodontal membrane was included. (3) Foreign bodies such as fragments of dental filling material were not included. While about one section in 25 of gingivae contained an inclusion, no idea as to the frequency of occurrence of such bodies in normal gingivae was obtained since the bodies were found only in granulation tissue that was usually old and hyalinized.

Pathological calcification in the gingivae undoubtedly occurs under the same conditions as it does in the other tissues. Barr [2] has summarized the local conditions which result in pathological calcification as: alkalinity of the tissue, the formation of phosphoric acid in the tissue, and the combination of calcium with protein. There are also general factors which may bring about pathological calcification: elevated parathyroid hormone levels, prolonged renal failure, and rapid bone resorption. Pathological calcification is therefore to be regarded as a biochemical process operating locally or generally which manifests itself by morphological changes that are frequently visible microscopically.

The first change observed in the formation of these calcified bodies was the transition of hyalinized connective tissues into rounded, homo-

* Received for publication, November 6, 1945.
† Deceased.

geneous masses. This is shown in Figure 1. Such rounded masses already contain calcium (Fig. 2). Clumping or agglutination of these "spheroids" leads to the formation of the larger calcified masses (Figs. 2, 4, and 5). After the calcified masses agglutinate, they become more dense (Figs. 6 and 7). By examining only large, rounded, blue-staining masses such as are shown in Figure 8, it is impossible to tell whether they originated by fragmentation of dental structures and are "dentin-cementum remnants," or whether they came about by the slow deposition of calcium. But after examining earlier and smaller bodies, one is forced to conclude that such bodies originate by infiltration of calcium salts, rather than by fragmentation of teeth. That the structures in Figure 8 are not remnants of dental filling material is shown by two facts. There are structures of various sizes present in the immediate area representing different stages, and there is an internal structure visible under strong light.

Fibroblasts in long and intimate association with calcium salts may acquire bone-forming properties. Because of this metaplasia, bone is often seen in calcium deposits in the eye, the walls of arteries, and in old areas of inflammation. Usually the transformation of calcium deposits into bone is by endochondral ossification.[3] Bone probably forms in areas of pathological calcification of the gingivae, as occasionally imperfectly forming bone was found close to areas of calcified nodules (Fig. 9).

Summary

There are, then, two views as to the origin of round calcified bodies such as those shown in Figure 8. Orban has explained them as resulting directly from a fragmentation of the dental structures; that is, as being "dentin-cementum remnants." The view set forth in this paper is that they result from calcified deposits in necrotic products of chronic inflammation of gingival tissues. If one examines only the end-product such as the large and fully calcified body (Fig. 8), it is impossible to determine whether it developed by fragmentation of dental remnants or by a process of pathological calcification. That the latter method of formation is possible is demonstrated in this study. It is also evident that ossification sometimes follows pathological calcification in gingival tissue.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 270

All sections were stained with hematoxylin and eosin except Figure 2. (Photomicrographs by Dr. Merrill Shepro.)

FIG. 1. Photomicrograph of gingivae showing the transition of dense hyalinized connective tissue, A, to spheroids of pathological calcifications, B and C. × 490.

FIG. 2. Calcium is present during the spheroid stage, as shown by von Kossa's stain for calcium. × 880.

FIG. 3. "Drawing together" or agglutination of the spheroids to form clumps. This is shown particularly at A and B. × 100.
Figures 1, 2, and 3 are from the same specimen.

FIG. 4. Clumping, A, B, and C, of calcareous bodies as shown in another specimen of chronically inflamed tissue. × 650.

FIG. 5. Clumping results in basophilic rounding bodies shown at A, B, C, and D. E is the area shown in Figure 4. × 200.

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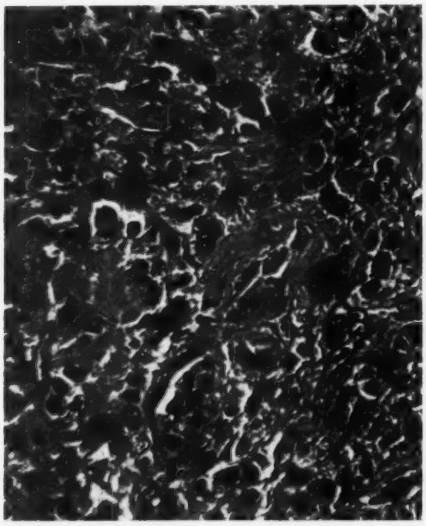

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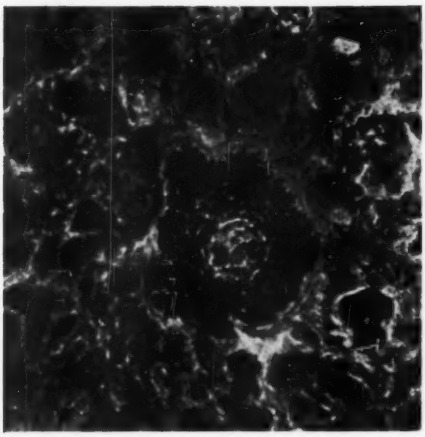

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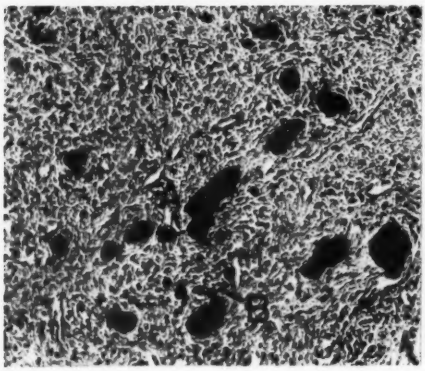

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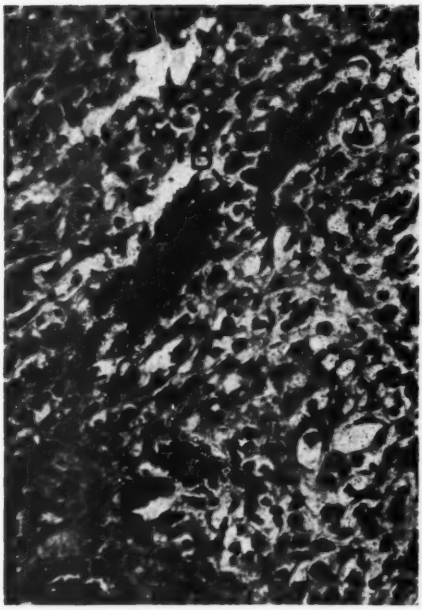

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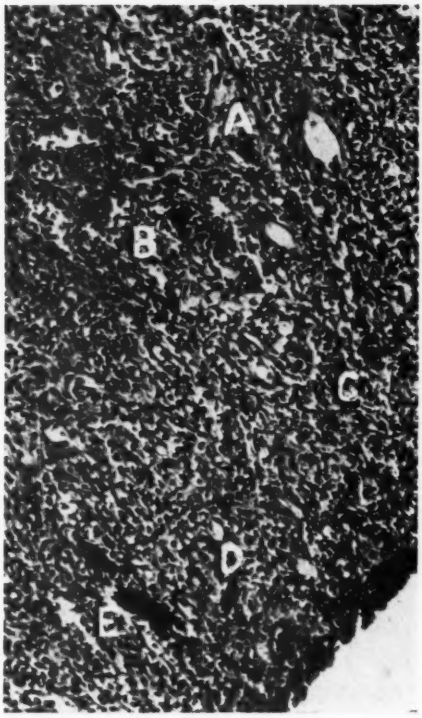

PLATE 271

FIG. 6. The rounding bodies become dense, as shown at A and B. Some have a concentric arrangement, C and D. × 200.

FIG. 7. The pathological calcareous bodies here resemble bone. This tissue was taken from a site just opposite (on the anterior surface of the alveolar bone) that from which Figures 1, 2, and 3 were obtained. × 300.

FIG. 8. The calcareous bodies become large and extremely dense. These have been termed "dentin-cementum remnants."[1] × 500.

FIG. 9. Ossification sometimes follows pathological calcification. This area of osteogenesis was found in the same specimen, and close to, the bodies shown in Figures 4 and 5. × 440.

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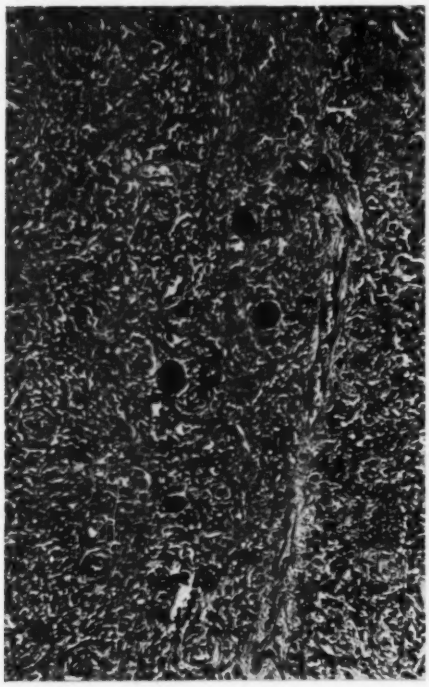

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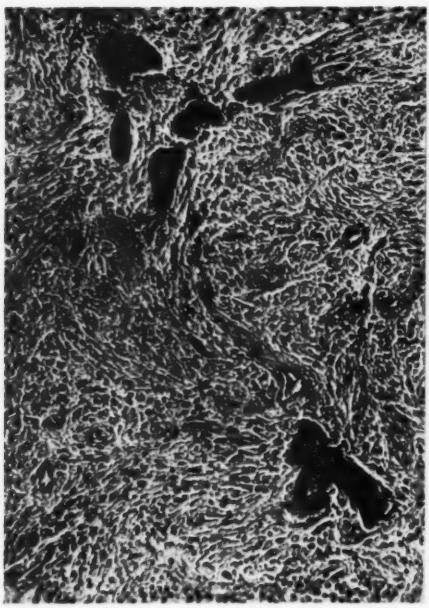

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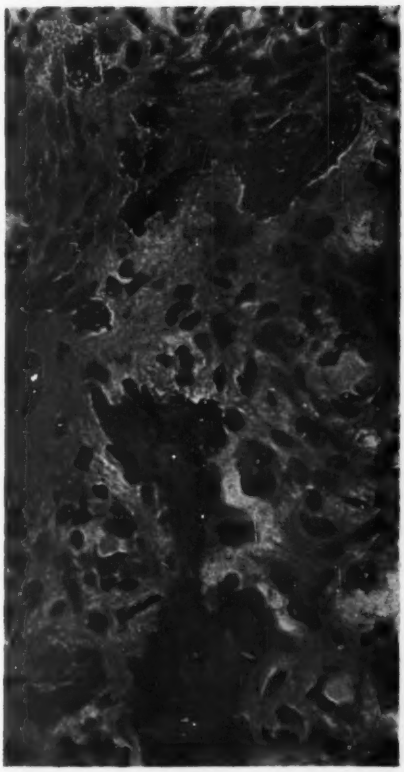

Calcification in the Gingivae

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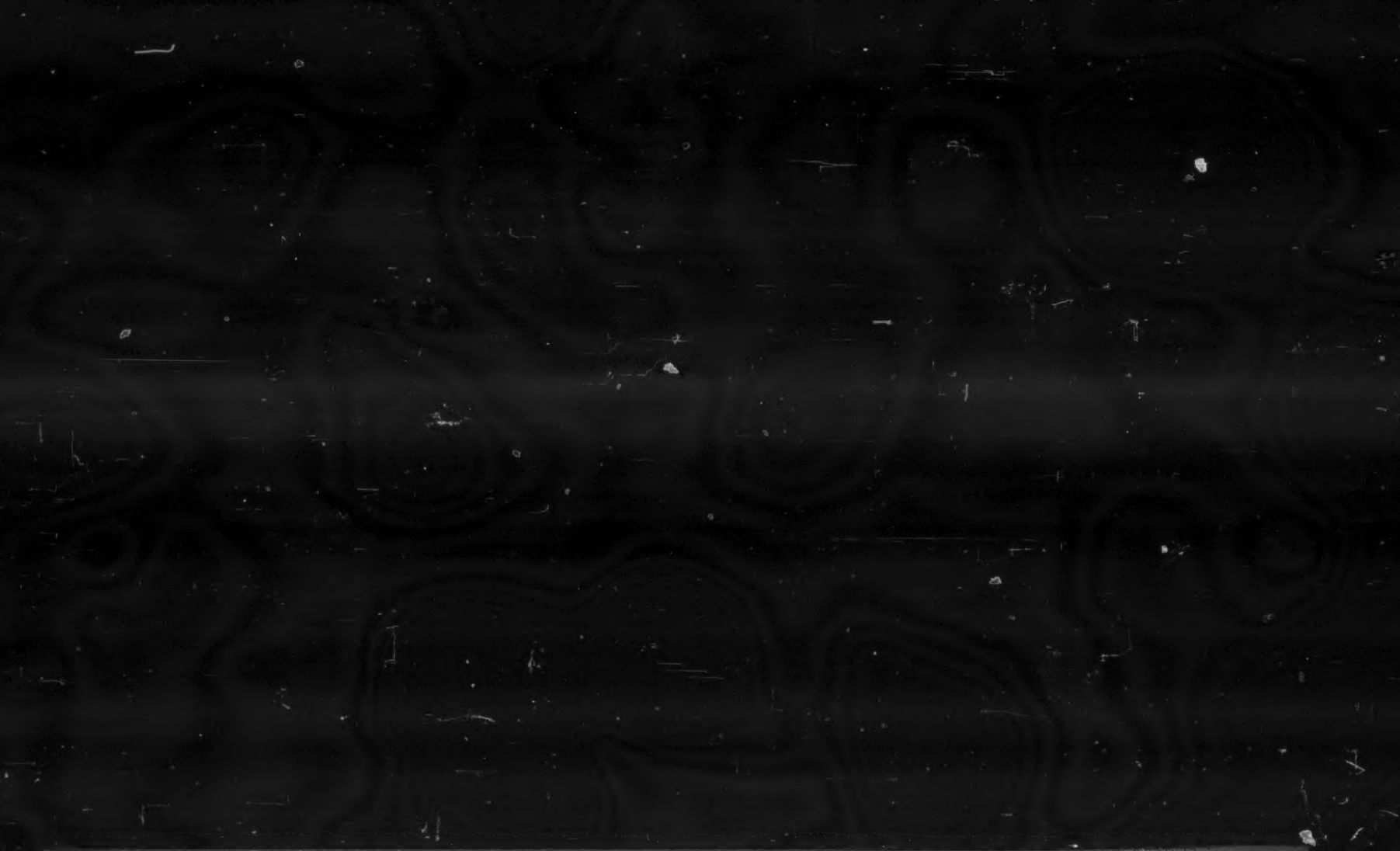
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